Assay Theory

The Z´-LYTE™ biochemical assay employs a fluorescence-based, coupled-enzyme format and is based on the differential sensitivity of phosphorylated and non-phosphorylated peptides to proteolytic cleavage (Figure 1). The peptide substrate is labeled with two fluorophores—one at each end—that make up a FRET pair.

In the primary reaction, the kinase transfers the gamma-phosphate of ATP to a single tyrosine, serine or threonine residue in a synthetic FRET-peptide. In the secondary reaction, a site-specific protease recognizes and cleaves non-phosphorylated FRET-peptides. Phosphorylation of FRET-peptides suppresses cleavage by the Development Reagent. Cleavage disrupts FRET between the donor (i.e., coumarin) and acceptor (i.e., fluorescein) fluorophores on the FRET-peptide, whereas uncleaved, phosphorylated FRET-peptides maintain FRET. A ratiometric method, which calculates the ratio (the Emission Ratio) of donor emission to acceptor emission after excitation of the donor fluorophore at 400 nm, is used to quantitate reaction progress, as shown in the equation below.

$$\text{Emission Ratio} = \frac{\text{Coumarin Emission (445 nm)}}{\text{Fluorescein Emission (520 nm)}}$$

A significant benefit of this ratiometric method for quantitating reaction progress is the elimination of well-to-well variations in FRET-peptide concentration and signal intensities. As a result, the assay yields very high Z´-factor values (>0.7) at a low percent phosphorylation.

Both cleaved and uncleaved FRET-peptides contribute to the fluorescence signals and therefore to the Emission Ratio. The extent of phosphorylation of the FRET-peptide can be calculated from the Emission Ratio. The Emission Ratio will remain low if the FRET-peptide is phosphorylated (i.e., no kinase inhibition) and will be high if the FRET-peptide is non-phosphorylated (i.e., kinase inhibition).

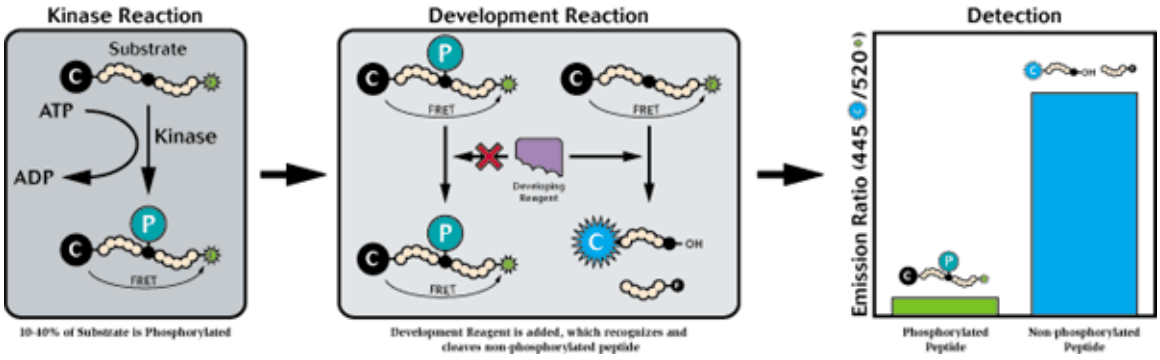

Figure 1: Z'-LYTE™ Illustration

SelectScreen™ Assay Conditions

Test Compounds
All Test Compounds are initially diluted to a 100X concentration in 100% DMSO. The 100X concentration is then diluted to a 4X working concentration in Kinase Buffer (50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM $MgCl_2$, 1 mM EGTA). The Test Compounds are in 1% DMSO final in the well.

Peptide/Kinase Mixtures
All Peptide/Kinase Mixtures are diluted to a 2X working concentration in the appropriate Kinase Buffer (see section *Kinase Specific Assay Conditions* for a complete description).

ATP Solution
All ATP Solutions are diluted to a 4X working concentration in Kinase Buffer (50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM $MgCl_2$, 1 mM EGTA).

Development Reagent Solution
The Development Reagent is diluted in Development Buffer (see section *Kinase-Specific Assay Conditions - Direct and Cascade* for a complete description).

Assay Protocol
1. 2.5 µL of the 4X Test Compound Solution is added to a barcoded Corning, low volume NBS, 384-well plate (Corning Cat. # 3676).
2. 5 µL of the 2X Peptide/Kinase Mixture is added to the plate.
3. 2.5 µL of the 4X ATP Solution is added to the plate.
4. Assay plate is shaken on a plate shaker for 30 seconds.
5. Assay plate is incubated for 60 minutes at room temperature.
6. 5 µL of the Development Reagent Solution is added to the plate.
7. Assay plate is shaken on a plate shaker for 30 seconds.
8. Assay plate is incubated for 60 minutes at room temperature.
9. Assay plate is read on fluorescence plate reader and the data is analyzed.

SelectScreen™ Assay Controls

The following controls are made for each individual kinase and are located on the same plate as the kinase:

0% Phosphorylation Control (100% Inhibition Control)
The maximum Emission Ratio is established by the 0% Phosphorylation Control (100% Inhibition Control), which contains no ATP and therefore exhibits no kinase activity. This control yields 100% cleaved peptide in the Development Reaction.

100% Phosphorylation Control
The 100% Phosphorylation Control, which consists of a synthetically phosphorylated peptide of the same sequence as the peptide substrate, is designed to allow for the calculation of percent phosphorylation. This control yields a very low percentage of cleaved peptide in the Development Reaction.

The 0% Phosphorylation and 100% Phosphorylation Controls allow one to calculate the percent Phosphorylation achieved in a specific reaction well. Control wells do not include any kinase inhibitors.

0% Inhibition Control
The minimum Emission Ratio in a screen is established by the 0% Inhibition Control, which contains active kinase. This control is designed to produce a 10–50% phosphorylated peptide in the Kinase Reaction and to yield 60–80% cleaved peptide in the Development Reaction.

Known Inhibitor
A known inhibitor control standard curve, 10 point titration, is run for each individual kinase on the same plate as the kinase to ensure the kinase is inhibited within an expected IC_{50} range previously determined as well as R^2 and Hillslope values within appropriate ranges.

The following controls are prepared for each concentration of Test Compound assayed:

Development Reaction Interference
The Development Reaction Interference is established by comparing the Test Compound Control wells that do not contain ATP versus the 0% Phosphorylation Control (which does not contain the Test Compound).

Test Compound Fluorescence Interference
The Test Compound Fluorescence Interference is determined by comparing the Test Compound Control wells that do not contain the Kinase/Peptide Mixture (zero peptide control) versus the 0% Inhibition Control.

SelectScreen™ Data Analysis

The following equations are used for each set of data points:

	Equation
Correction for Background Fluorescence	$FI_{Sample} - FI_{TCFI\ Ctl}$
Emission Ratio (using values corrected for background fluorescence)	$\frac{\text{Coumarin Emission (445 nm)}}{\text{Fluorescein Emission (520 nm)}}$
% Phosphorylation (% Phos)	$\left\{ 1 - \frac{(\text{Emission Ratio} \times F_{100\%}) - C_{100\%}}{(C_{0\%} - C_{100\%}) + [\text{Emission Ratio} \times (F_{100\%} - F_{0\%})]} \right\} * 100$
% Inhibition	$\left\{ 1 - \frac{\%\ Phos_{\ Sample}}{\%\ Phos_{\ 0\%\ Inhibition\ Ctl}} \right\} * 100$
Z′ (using Emission Ratio values)	$1 - \frac{3*Stdev_{\ 0\%\ Phos\ Ctl} + 3*Stdev_{\ 0\%\ Inhibition}}{Mean_{\ 0\%Phos\ Ctl} - Mean_{\ 0\%\ Inhibition}}$
Difference Between Data Points (single point only)	$\lvert\ \%\ Inhibition_{\ Point\ 1} - \%\ Inhibition_{\ Point\ 2}\ \rvert$
Development Reaction Interference (DRI) (no ATP control)	$\frac{Emission\ Ratio_{\ DRI\ Ctl}}{Emission\ Ratio_{\ 0\%\ Phos\ Ctl}}$
Test Compound Fluorescence Interference (TCFI) (check both Coumarin and Fluorescein emissions)	$\frac{FI_{\ TCFI\ Ctl}}{FI_{\ 0\%\ Inhibitor\ Ctl}}$

FI = Fluorescence Intensity
$C_{100\%}$ = Average Coumarin emission signal of the 100% Phos. Control
$C_{0\%}$ = Average Coumarin emission signal of the 0% Phos. Control
$F_{100\%}$ = Average Fluorescein emission signal of the 100% Phos. Control
$F_{0\%}$ = Average Fluorescein emission signal of the 0% Phos. Control
DRI = Development Reaction Interference
TCFI = Test Compound Fluorescence Intereference

Graphing Software

SelectScreen™ Kinase Profiling Service uses XL*fit* from IDBS. The dose response curve is curve fit to model number 205 (sigmoidal dose-response model). If the bottom of the curve does not fit between -20% & 20% inhibition, it is set to 0% inhibition. If the top of the curve does not fit between 70% and 130% inhibition, it is set to 100% inhibition.

Kinase-Specific Assay Conditions – Direct Format

ABL1
The 2X ABL1 / Tyr 02 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.2 - 1.9 ng ABL1 and 2 µM Tyr 02 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

ABL1 E255K
The 2X ABL1 E255K / Tyr 02 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.8 - 5.2 ng ABL1 E255K and 2 µM Tyr 02 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

ABL1 G250E
The 2X ABL1 G250E / Tyr 02 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.8 - 7.9 ng ABL1 G250E and 2 µM Tyr 02 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

ABL1 T315I
The 2X ABL1 T315I / Tyr 02 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.4 - 8.2 ng ABL1 T315I and 2 µM Tyr 02 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

ABL1 Y253F
The 2X ABL1 Y253F / Tyr 02 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.7 - 5.2 ng ABL1 Y253F and 2 µM Tyr 02 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

ABL2 (Arg)
The 2X ABL2 (Arg) / Tyr 02 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.4 - 5.3 ng ABL2 (Arg) and 2 µM Tyr 02 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

ACVR1B (ALK4)
The 2X ACVR1B (ALK4) / Ser/Thr 16 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MnCl2, 1 mM EGTA, 2 mM DTT, 0.02% NaN3. The final 10 µL Kinase Reaction consists of 10.0 - 62.2 ng ACVR1B (ALK4) and 2 µM Ser/Thr 16 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 5 mM MgCl2, 5 mM MnCl2, 1 mM EGTA, 1 mM DTT, 0.01% NaN3. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:8 dilution of Development Reagent B is added.

ADRBK1 (GRK2)
The 2X ADRBK1 (GRK2) / Ser/Thr 16 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 11.0 - 121.2 ng ADRBK1 (GRK2) and 2 µM Ser/Thr 16 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:8 dilution of Development Reagent B is added.

ADRBK2 (GRK3)
The 2X ADRBK2 (GRK3) / Ser/Thr 16 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 9.9 - 89.0 ng ADRBK2 (GRK3) and 2 µM Ser/Thr 16 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:8 dilution of Development Reagent B is added.

AKT1 (PKB alpha)
The 2X AKT1 (PKB alpha) / Ser/Thr 06 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.5 - 8.6 ng AKT1 (PKB alpha) and 2 µM Ser/Thr 06 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:2048 dilution of Development Reagent A is added.

AKT2 (PKB beta)
The 2X AKT2 (PKB beta) / Ser/Thr 06 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.0 - 40.0 ng AKT2 (PKB beta) and 2 µM Ser/Thr 06 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:2048 dilution of Development Reagent A is added.

AKT3 (PKB gamma)
The 2X AKT3 (PKB gamma) / Ser/Thr 06 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.4 - 8.3 ng AKT3 (PKB gamma) and 2 µM Ser/Thr 06 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:2048 dilution of Development Reagent A is added.

ALK
The 2X ALK / Tyr 01 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 20.3 - 243.5 ng ALK and 2 µM Tyr 01 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:128 dilution of Development Reagent B is added.

AURKB (Aurora B)
The 2X AURKB (Aurora B) / Ser/Thr 01 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 3.7 - 196.5 ng AURKB (Aurora B) and 2 µM Ser/Thr 01 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:2048 dilution of Development Reagent A is added.

AURKC (Aurora C)
The 2X AURKC (Aurora C) / Ser/Thr 19 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 6.5 - 103.1 ng AURKC (Aurora C) and 2 µM Ser/Thr 19 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:256 dilution of Development Reagent A is added.

BLK
The 2X BLK / Tyr 01 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.5 - 4.5 ng BLK and 2 µM Tyr 01 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:128 dilution of Development Reagent B is added.

BMX
The 2X BMX / Tyr 01 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2.8 - 85.0 ng BMX and 2 µM Tyr 01 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:128 dilution of Development Reagent B is added.

BRSK1 (SAD1)
The 2X BRSK1 (SAD1) / Ser/Thr 21 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2.8 - 41.7 ng BRSK1 (SAD1) and 2 µM Ser/Thr 21 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

BTK
The 2X BTK / Tyr 01 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.0 - 15.0 ng BTK and 2 µM Tyr 01 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:128 dilution of Development Reagent B is added.

CAMK1D (CaMKI delta)
The 2X CAMK1D (CaMKI delta) / Ser/Thr 10 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 4 mM CaCl2, 40 µg/ml Calmodulin, 0.02% NaN3. The final 10 µL Kinase Reaction consists of 2.1 - 40.0 ng CAMK1D (CaMKI delta) and 2 µM Ser/Thr 10 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 500 µM EGTA, 2 mM CaCl2, 20 µg/ml Calmodulin, 0.01% NaN3. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1500 dilution of Development Reagent A is added.

CAMK2A (CaMKII alpha)
The 2X CAMK2A (CaMKII alpha) / Ser/Thr 04 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 4 mM CaCl2, 40 µg/ml Calmodulin, 0.02% NaN3. The final 10 µL Kinase Reaction consists of 0.1 - 1.3 ng CAMK2A (CaMKII alpha) and 2 µM Ser/Thr 04 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 500 µM EGTA, 2 mM CaCl2, 20 µg/ml Calmodulin, 0.01% NaN3. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

CAMK2B (CaMKII beta)
The 2X CAMK2B (CaMKII beta) / Ser/Thr 17 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 4 mM CaCl2, 40 µg/ml Calmodulin, 0.02% NaN3. The final 10 µL Kinase Reaction consists of 0.7 - 16.6 ng CAMK2B (CaMKII beta) and 2 µM Ser/Thr 17 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 500 µM EGTA, 2 mM CaCl2, 20 µg/ml Calmodulin, 0.01% NaN3. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

CAMK2D (CaMKII delta)
The 2X CAMK2D (CaMKII delta) / Ser/Thr 04 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 4 mM CaCl2, 40 µg/ml Calmodulin, 0.02% NaN3. The final 10 µL Kinase Reaction consists of 0.1 - 0.8 ng CAMK2D (CaMKII delta) and 2 µM Ser/Thr 04 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 500 µM EGTA, 2 mM CaCl2, 20 µg/ml Calmodulin, 0.01% NaN3. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

CAMK4 (CaMKIV)
The 2X CAMK4 (CaMKIV) / Ser/Thr 13 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 4 mM CaCl2, 40 µg/ml Calmodulin, 0.02% NaN3. The final 10 µL Kinase Reaction consists of 6.3 - 62.8 ng CAMK4 (CaMKIV) and 2 µM Ser/Thr 13 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 500 µM EGTA, 2 mM CaCl2, 20 µg/ml Calmodulin, 0.01% NaN3. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:512 dilution of Development Reagent A is added.

CDC42 BPA (MRCKA)
The 2X CDC42 BPA (MRCKA) / Ser/Thr 13 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 9.5 - 68.2 ng CDC42 BPA (MRCKA) and 2 µM Ser/Thr 13 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:512 dilution of Development Reagent A is added.

CDC42 BPB (MRCKB)
The 2X CDC42 BPB (MRCKB) / Ser/Thr 13 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 3.6 - 15.0 ng CDC42 BPB (MRCKB) and 2 µM Ser/Thr 13 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:512 dilution of Development Reagent A is added.

CDK1/cyclin B
The 2X CDK1/cyclin B / Ser/Thr 18 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2.0 - 21.4 ng CDK1/cyclin B and 2 µM Ser/Thr 18 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

CDK2/cyclin A
The 2X CDK2/cyclin A / Ser/Thr 12 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2.3 - 30.5 ng CDK2/cyclin A and 2 µM Ser/Thr 12 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:4096 dilution of Development Reagent A is added.

CDK5/p35
The 2X CDK5/p35 / Ser/Thr 12 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.2 - 1.5 ng CDK5/p35 and 2 µM Ser/Thr 12 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:4096 dilution of Development Reagent A is added.

CHEK1 (CHK1)
The 2X CHEK1 (CHK1) / Ser/Thr 07 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.3 - 20.0 ng CHEK1 (CHK1) and 2 µM Ser/Thr 07 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

CHEK2 (CHK2)
The 2X CHEK2 (CHK2) / Ser/Thr 07 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 6.0 - 400.0 ng CHEK2 (CHK2) and 2 µM Ser/Thr 07 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

CLK1
The 2X CLK1 / Ser/Thr 09 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 16.0 - 140.0 ng CLK1 and 2 µM Ser/Thr 09 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

CLK2
The 2X CLK2 / Ser/Thr 06 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.8 - 21.6 ng CLK2 and 2 µM Ser/Thr 06 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:2048 dilution of Development Reagent A is added.

CLK3
The 2X CLK3 / Ser/Thr 18 Peptide Mixture is prepared in 50 mM Tris pH 8.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA, 0.02% NaN3. The final 10 µL Kinase Reaction consists of 8.8 - 41.5 ng CLK3 and 2 µM Ser/Thr 18 Peptide in 50 mM Tris / HEPES pH 8.0, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA, 0.01% NaN3. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

CSF1R (FMS)
The 2X CSF1R (FMS) / Tyr 01 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.2 - 67.3 ng CSF1R (FMS) and 2 µM Tyr 01 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:128 dilution of Development Reagent B is added.

CSK
The 2X CSK / Tyr 02 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2.0 - 26.0 ng CSK and 2 µM Tyr 02 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

CSNK1A1 (CK1 alpha 1)
The 2X CSNK1A1 (CK1 alpha 1) / Ser/Thr 11 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 16.7 - 107.0 ng CSNK1A1 (CK1 alpha 1) and 2 µM Ser/Thr 11 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:16 dilution of Development Reagent B is added.

CSNK1D (CK1 delta)
The 2X CSNK1D (CK1 delta) / Ser/Thr 11 Peptide Mixture is prepared in 50 mM Tris pH 8.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA, 0.02% NaN3. The final 10 µL Kinase Reaction consists of 17.2 - 138.2 ng CSNK1D (CK1 delta) and 2 µM Ser/Thr 11 Peptide in 50 mM Tris / HEPES pH 8.0, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA, 0.01% NaN3. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:16 dilution of Development Reagent B is added.

CSNK1E (CK1 epsilon)
The 2X CSNK1E (CK1 epsilon) / Ser/Thr 11 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 6.0 - 40.0 ng CSNK1E (CK1 epsilon) and 2 µM Ser/Thr 11 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:16 dilution of Development Reagent B is added.

CSNK1G1 (CK1 gamma 1)
The 2X CSNK1G1 (CK1 gamma 1) / Ser/Thr 05 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 3.7 - 19.5 ng CSNK1G1 (CK1 gamma 1) and 2 µM Ser/Thr 05 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:128 dilution of Development Reagent B is added.

CSNK1G2 (CK1 gamma 2)
The 2X CSNK1G2 (CK1 gamma 2) / Ser/Thr 05 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.5 - 16.5 ng CSNK1G2 (CK1 gamma 2) and 2 µM Ser/Thr 05 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:128 dilution of Development Reagent B is added.

CSNK1G3 (CK1 gamma 3)
The 2X CSNK1G3 (CK1 gamma 3) / Ser/Thr 05 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 4.3 - 31.7 ng CSNK1G3 (CK1 gamma 3) and 2 µM Ser/Thr 05 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:128 dilution of Development Reagent B is added.

CSNK2A1 (CK2 alpha 1)
The 2X CSNK2A1 (CK2 alpha 1) / Ser/Thr 11 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2.8 - 22.5 ng CSNK2A1 (CK2 alpha 1) and 2 µM Ser/Thr 11 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:16 dilution of Development Reagent B is added.

CSNK2A2 (CK2 alpha 2)
The 2X CSNK2A2 (CK2 alpha 2) / Ser/Thr 11 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 3.7 - 26.3 ng CSNK2A2 (CK2 alpha 2) and 2 µM Ser/Thr 11 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:16 dilution of Development Reagent B is added.

DAPK3 (ZIPK)
The 2X DAPK3 (ZIPK) / Ser/Thr 13 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 11.6 - 80.0 ng DAPK3 (ZIPK) and 2 µM Ser/Thr 13 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:512 dilution of Development Reagent A is added.

DCAMKL2 (DCK2)
The 2X DCAMKL2 (DCK2) / Ser/Thr 17 Peptide Mixture is prepared in 50 mM HEPES pH 6.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA, 0.02% NaN3. The final 10 µL Kinase Reaction consists of 14.2 - 100.9 ng DCAMKL2 (DCK2) and 2 µM Ser/Thr 17 Peptide in 50 mM HEPES pH 7.0, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA, 0.01% NaN3. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

DYRK1A
The 2X DYRK1A / Ser/Thr 18 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.9 - 25.0 ng DYRK1A and 2 µM Ser/Thr 18 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

DYRK3
The 2X DYRK3 / Ser/Thr 09 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.4 - 10.9 ng DYRK3 and 2 µM Ser/Thr 09 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

DYRK4
The 2X DYRK4 / Ser/Thr 09 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 13.6 - 103.6 ng DYRK4 and 2 µM Ser/Thr 09 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

EGFR (ErbB1)
The 2X EGFR (ErbB1) / Tyr 04 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 4 mM MnCl2, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 1.3 - 7.9 ng EGFR (ErbB1) and 2 µM Tyr 04 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 2 mM MnCl2, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32 dilution of Development Reagent B is added.

EGFR L858R (ErbB1 L858R)
The 2X EGFR L858R (ErbB1 L858R) / Tyr 04 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 4 mM MnCl2, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 0.6 - 4.5 ng EGFR L858R (ErbB1 L858R) and 2 µM Tyr 04 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 2 mM MnCl2, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32 dilution of Development Reagent B is added.

EGFR L861Q (ErbB1 L861Q)
The 2X EGFR L861Q (ErbB1 L861Q) / Tyr 04 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 4 mM MnCl2, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 1.2 - 8.4 ng EGFR L861Q (ErbB1 L861Q) and 2 µM Tyr 04 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 2 mM MnCl2, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32 dilution of Development Reagent B is added.

EPHA1
The 2X EPHA1 / Tyr 02 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 5.5 - 60.7 ng EPHA1 and 2 µM Tyr 02 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

EPHA2
The 2X EPHA2 / Tyr 01 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2.1 - 26.2 ng EPHA2 and 2 µM Tyr 01 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:128 dilution of Development Reagent B is added.

EPHA3
The 2X EPHA3 / Tyr 01 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2.3 - 145.5 ng EPHA3 and 2 µM Tyr 01 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:128 dilution of Development Reagent B is added.

EPHA4
The 2X EPHA4 / Tyr 02 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 5.8 - 23.0 ng EPHA4 and 2 µM Tyr 02 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

EPHA5
The 2X EPHA5 / Tyr 01 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.5 - 38.1 ng EPHA5 and 2 µM Tyr 01 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:128 dilution of Development Reagent B is added.

EPHA8
The 2X EPHA8 / Tyr 02 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 3.2 - 49.1 ng EPHA8 and 2 µM Tyr 02 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

EPHB1
The 2X EPHB1 / Tyr 02 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2.4 - 63.1 ng EPHB1 and 2 µM Tyr 02 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

EPHB2
The 2X EPHB2 / Tyr 02 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.7 - 18.0 ng EPHB2 and 2 µM Tyr 02 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

EPHB3
The 2X EPHB3 / Tyr 02 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2.0 - 43.8 ng EPHB3 and 2 µM Tyr 02 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

EPHB4
The 2X EPHB4 / Tyr 01 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.3 - 18.0 ng EPHB4 and 2 µM Tyr 01 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:128 dilution of Development Reagent B is added.

ERBB2 (HER2)
The 2X ERBB2 (HER2) / Tyr 06 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MnCl2, 1 mM EGTA, 2 mM DTT, 0.02% NaN3. The final 10 µL Kinase Reaction consists of 4.1 - 34.1 ng ERBB2 (HER2) and 2 µM Tyr 06 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 5 mM MgCl2, 5 mM MnCl2, 1 mM EGTA, 1 mM DTT, 0.01% NaN3. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

ERBB4 (HER4)
The 2X ERBB4 (HER4) / Tyr 01 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 4 mM MnCl2, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 2.0 - 23.0 ng ERBB4 (HER4) and 2 µM Tyr 01 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 2 mM MnCl2, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:128 dilution of Development Reagent B is added.

FER
The 2X FER / Tyr 05 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.6 - 21.7 ng FER and 2 µM Tyr 05 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

FES (FPS)
The 2X FES (FPS) / Tyr 01 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.7 - 5.2 ng FES (FPS) and 2 µM Tyr 01 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:128 dilution of Development Reagent B is added.

FGFR1
The 2X FGFR1 / Tyr 04 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 4 mM MnCl2, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 0.4 - 3.7 ng FGFR1 and 2 µM Tyr 04 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 2 mM MnCl2, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32 dilution of Development Reagent B is added.

FGFR2
The 2X FGFR2 / Tyr 04 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 4 mM MnCl2, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 0.3 - 2.9 ng FGFR2 and 2 µM Tyr 04 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 2 mM MnCl2, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32 dilution of Development Reagent B is added.

FGFR3
The 2X FGFR3 / Tyr 04 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 4 mM MnCl2, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 1.5 - 20.0 ng FGFR3 and 2 µM Tyr 04 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 2 mM MnCl2, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32 dilution of Development Reagent B is added.

FGFR3 K650E
The 2X FGFR3 K650E / Tyr 04 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 4 mM MnCl2, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 0.6 - 4.7 ng FGFR3 K650E and 2 µM Tyr 04 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 2 mM MnCl2, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32 dilution of Development Reagent B is added.

FGFR4
The 2X FGFR4 / Tyr 04 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 4 mM MnCl2, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 2.4 - 105.0 ng FGFR4 and 2 µM Tyr 04 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 2 mM MnCl2, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32 dilution of Development Reagent B is added.

FGR
The 2X FGR / Tyr 02 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.8 - 6.0 ng FGR and 2 µM Tyr 02 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

FLT1 (VEGFR1)
The 2X FLT1 (VEGFR1) / Tyr 04 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 4 mM MnCl2, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 5.0 - 25.0 ng FLT1 (VEGFR1) and 2 µM Tyr 04 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 2 mM MnCl2, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32 dilution of Development Reagent B is added.

FLT3
The 2X FLT3 / Tyr 02 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.6 - 76.0 ng FLT3 and 2 µM Tyr 02 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

FLT3 D835Y
The 2X FLT3 D835Y / Tyr 02 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.1 - 1.3 ng FLT3 D835Y and 2 µM Tyr 02 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

FLT4 (VEGFR3)
The 2X FLT4 (VEGFR3) / Tyr 04 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 4 mM MnCl2, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 1.9 - 16.0 ng FLT4 (VEGFR3) and 2 µM Tyr 04 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 2 mM MnCl2, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32 dilution of Development Reagent B is added.

FRK (PTK5)
The 2X FRK (PTK5) / Tyr 01 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.1 - 51.9 ng FRK (PTK5) and 2 µM Tyr 01 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:128 dilution of Development Reagent B is added.

FYN
The 2X FYN / Tyr 02 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 16.6 - 132.1 ng FYN and 2 µM Tyr 02 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

GRK4
The 2X GRK4 / Ser/Thr 16 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 4.0 - 19.1 ng GRK4 and 2 µM Ser/Thr 16 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:8 dilution of Development Reagent B is added.

GRK5
The 2X GRK5 / Ser/Thr 16 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 4 mM MnCl2, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 12.1 - 97.5 ng GRK5 and 2 µM Ser/Thr 16 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 2 mM MnCl2, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:8 dilution of Development Reagent B is added.

GRK6
The 2X GRK6 / Ser/Thr 16 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 9.1 - 82.9 ng GRK6 and 2 µM Ser/Thr 16 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:8 dilution of Development Reagent B is added.

GRK7
The 2X GRK7 / Ser/Thr 16 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 3.8 - 21.4 ng GRK7 and 2 µM Ser/Thr 16 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:8 dilution of Development Reagent B is added.

GSK3A (GSK3 alpha)
The 2X GSK3A (GSK3 alpha) / Ser/Thr 09 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.1 - 0.4 ng GSK3A (GSK3 alpha) and 2 µM Ser/Thr 09 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

GSK3B (GSK3 beta)
The 2X GSK3B (GSK3 beta) / Ser/Thr 09 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.4 - 2.5 ng GSK3B (GSK3 beta) and 2 µM Ser/Thr 09 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

HCK
The 2X HCK / Tyr 02 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.3 - 3.5 ng HCK and 2 µM Tyr 02 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

HIPK1 (Myak)
The 2X HIPK1 (Myak) / Ser/Thr 09 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.7 - 24.0 ng HIPK1 (Myak) and 2 µM Ser/Thr 09 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

HIPK4
The 2X HIPK4 / Ser/Thr 18 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 10.9 - 88.9 ng HIPK4 and 2 µM Ser/Thr 18 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

IGF1R
The 2X IGF1R / Tyr 01 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 3.8 - 98.8 ng IGF1R and 2 µM Tyr 01 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:128 dilution of Development Reagent B is added.

IKBKB (IKK beta)
The 2X IKBKB (IKK beta) / Ser/Thr 05 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.2 - 10.6 ng IKBKB (IKK beta) and 2 µM Ser/Thr 05 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:128 dilution of Development Reagent B is added.

INSR
The 2X INSR / Tyr 01 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 4 mM MnCl2, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 2.3 - 50.0 ng INSR and 2 µM Tyr 01 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 2 mM MnCl2, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:128 dilution of Development Reagent B is added.

INSRR (IRR)
The 2X INSRR (IRR) / Tyr 04 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 4 mM MnCl2, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 2.2 - 14.7 ng INSRR (IRR) and 2 µM Tyr 04 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 2 mM MnCl2, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32 dilution of Development Reagent B is added.

IRAK4
The 2X IRAK4 / Ser/Thr 07 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MnCl2, 1 mM EGTA, 2 mM DTT, 0.02% NaN3. The final 10 µL Kinase Reaction consists of 3.0 - 42.8 ng IRAK4 and 2 µM Ser/Thr 07 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 5 mM MgCl2, 5 mM MnCl2, 1 mM EGTA, 1 mM DTT, 0.01% NaN3. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

ITK
The 2X ITK / Tyr 01 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 4.5 - 90.0 ng ITK and 2 µM Tyr 01 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:128 dilution of Development Reagent B is added.

JAK2
The 2X JAK2 / Tyr 06 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 4 mM MnCl2, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 0.3 - 2.3 ng JAK2 and 2 µM Tyr 06 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 2 mM MnCl2, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

JAK2 JH1 JH2
The 2X JAK2 JH1 JH2 / Tyr 06 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 4.8 - 57.8 ng JAK2 JH1 JH2 and 2 µM Tyr 06 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

JAK3
The 2X JAK3 / Tyr 04 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 4 mM MnCl2, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 6.7 - 49.1 ng JAK3 and 2 µM Tyr 04 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 2 mM MnCl2, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32 dilution of Development Reagent B is added.

KDR (VEGFR2)
The 2X KDR (VEGFR2) / Tyr 01 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.6 - 14.4 ng KDR (VEGFR2) and 2 µM Tyr 01 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:128 dilution of Development Reagent B is added.

KIT
The 2X KIT / Tyr 06 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MnCl2, 1 mM EGTA, 2 mM DTT, 0.02% NaN3. The final 10 µL Kinase Reaction consists of 3.7 - 27.7 ng KIT and 2 µM Tyr 06 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 5 mM MgCl2, 5 mM MnCl2, 1 mM EGTA, 1 mM DTT, 0.01% NaN3. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

KIT T670I
The 2X KIT T670I / Tyr 06 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MnCl2, 1 mM EGTA, 2 mM DTT, 0.02% NaN3. The final 10 µL Kinase Reaction consists of 4.8 - 127.0 ng KIT T670I and 2 µM Tyr 06 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 5 mM MgCl2, 5 mM MnCl2, 1 mM EGTA, 1 mM DTT, 0.01% NaN3. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

LCK
The 2X LCK / Tyr 02 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2.0 - 26.5 ng LCK and 2 µM Tyr 02 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

LYN A
The 2X LYN A / Tyr 02 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.1 - 12.0 ng LYN A and 2 µM Tyr 02 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

LYN B
The 2X LYN B / Tyr 02 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.9 - 18.5 ng LYN B and 2 µM Tyr 02 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

MAP3K9 (MLK1)
The 2X MAP3K9 (MLK1) / Ser/Thr 07 Peptide Mixture is prepared in 50 mM HEPES pH 6.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA, 0.02% NaN3. The final 10 µL Kinase Reaction consists of 12.0 - 55.6 ng MAP3K9 (MLK1) and 2 µM Ser/Thr 07 Peptide in 50 mM HEPES pH 7.0, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA, 0.01% NaN3. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

MAP4K2 (GCK)
The 2X MAP4K2 (GCK) / Ser/Thr 07 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.4 - 8.4 ng MAP4K2 (GCK) and 2 µM Ser/Thr 07 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

MAP4K4 (HGK)
The 2X MAP4K4 (HGK) / Ser/Thr 07 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.5 - 5.0 ng MAP4K4 (HGK) and 2 µM Ser/Thr 07 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

MAP4K5 (KHS1)
The 2X MAP4K5 (KHS1) / Ser/Thr 07 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.3 - 4.0 ng MAP4K5 (KHS1) and 2 µM Ser/Thr 07 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

MAPK1 (ERK2)
The 2X MAPK1 (ERK2) / Ser/Thr 03 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2.1 - 31.4 ng MAPK1 (ERK2) and 2 µM Ser/Thr 03 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

MAPK11 (p38 beta)
The 2X MAPK11 (p38 beta) / Ser/Thr 15 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 12.0 - 76.0 ng MAPK11 (p38 beta) and 2 µM Ser/Thr 15 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent B is added.

MAPK12 (p38 gamma)
The 2X MAPK12 (p38 gamma) / Ser/Thr 03 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.5 - 4.8 ng MAPK12 (p38 gamma) and 2 µM Ser/Thr 03 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

MAPK13 (p38 delta)
The 2X MAPK13 (p38 delta) / Ser/Thr 03 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 3.5 - 39.6 ng MAPK13 (p38 delta) and 2 µM Ser/Thr 03 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

MAPK3 (ERK1)
The 2X MAPK3 (ERK1) / Ser/Thr 03 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2.5 - 31.5 ng MAPK3 (ERK1) and 2 µM Ser/Thr 03 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

MAPKAPK2
The 2X MAPKAPK2 / Ser/Thr 04 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.1 - 0.5 ng MAPKAPK2 and 2 µM Ser/Thr 04 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

MAPKAPK3
The 2X MAPKAPK3 / Ser/Thr 04 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.8 - 15.8 ng MAPKAPK3 and 2 µM Ser/Thr 04 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

MAPKAPK5 (PRAK)
The 2X MAPKAPK5 (PRAK) / Ser/Thr 04 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2.1 - 18.0 ng MAPKAPK5 (PRAK) and 2 µM Ser/Thr 04 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

MARK1 (MARK)
The 2X MARK1 (MARK) / Ser/Thr 21 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 11.0 - 106.9 ng MARK1 (MARK) and 2 µM Ser/Thr 21 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

MARK2
The 2X MARK2 / Ser/Thr 21 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 10.3 - 180.0 ng MARK2 and 2 µM Ser/Thr 21 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

MATK (HYL)
The 2X MATK (HYL) / Tyr 01 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2.9 - 76.8 ng MATK (HYL) and 2 µM Tyr 01 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:128 dilution of Development Reagent B is added.

MERTK (cMER)
The 2X MERTK (cMER) / Tyr 02 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 4 mM MnCl2, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 0.7 - 7.8 ng MERTK (cMER) and 2 µM Tyr 02 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 2 mM MnCl2, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

MET (cMet)
The 2X MET (cMet) / Tyr 06 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.3 - 10.0 ng MET (cMet) and 2 µM Tyr 06 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

MET M1250T
The 2X MET M1250T / Tyr 06 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 3.0 - 30.0 ng MET M1250T and 2 µM Tyr 06 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

MINK1
The 2X MINK1 / Ser/Thr 07 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.2 - 35.0 ng MINK1 and 2 µM Ser/Thr 07 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

MST1R (RON)
The 2X MST1R (RON) / Tyr 06 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.1 - 1.2 ng MST1R (RON) and 2 µM Tyr 06 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

MST4
The 2X MST4 / Ser/Thr 07 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 10.5 - 89.8 ng MST4 and 2 µM Ser/Thr 07 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

MUSK
The 2X MUSK / Tyr 04 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 4 mM MnCl2, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 16.4 - 89.5 ng MUSK and 2 µM Tyr 04 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 2 mM MnCl2, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32 dilution of Development Reagent B is added.

MYLK2 (skMLCK)
The 2X MYLK2 (skMLCK) / Ser/Thr 13 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 4 mM CaCl2, 40 µg/ml Calmodulin, 0.02% NaN3. The final 10 µL Kinase Reaction consists of 5.8 - 24.4 ng MYLK2 (skMLCK) and 2 µM Ser/Thr 13 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 500 µM EGTA, 2 mM CaCl2, 20 µg/ml Calmodulin, 0.01% NaN3. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:512 dilution of Development Reagent A is added.

NEK1
The 2X NEK1 / Ser/Thr 07 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.9 - 69.1 ng NEK1 and 2 µM Ser/Thr 07 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

NEK2
The 2X NEK2 / Ser/Thr 07 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.4 - 4.0 ng NEK2 and 2 µM Ser/Thr 07 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

NEK4
The 2X NEK4 / Ser/Thr 17 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 7.4 - 39.3 ng NEK4 and 2 µM Ser/Thr 17 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

NTRK1 (TRKA)
The 2X NTRK1 (TRKA) / Tyr 01 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 5.1 - 77.4 ng NTRK1 (TRKA) and 2 µM Tyr 01 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:128 dilution of Development Reagent B is added.

NTRK2 (TRKB)
The 2X NTRK2 (TRKB) / Tyr 01 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 4 mM MnCl2, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 0.2 - 2.0 ng NTRK2 (TRKB) and 2 µM Tyr 01 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 2 mM MnCl2, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:128 dilution of Development Reagent B is added.

NTRK3 (TRKC)
The 2X NTRK3 (TRKC) / Tyr 01 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 3.3 - 110.6 ng NTRK3 (TRKC) and 2 µM Tyr 01 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:128 dilution of Development Reagent B is added.

PAK2 (PAK65)
The 2X PAK2 (PAK65) / Ser/Thr 20 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.1 - 4.5 ng PAK2 (PAK65) and 2 µM Ser/Thr 20 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:256 dilution of Development Reagent A is added.

PAK3
The 2X PAK3 / Ser/Thr 20 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.3 - 9.0 ng PAK3 and 2 µM Ser/Thr 20 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:256 dilution of Development Reagent A is added.

PAK4
The 2X PAK4 / Ser/Thr 20 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.1 - 0.6 ng PAK4 and 2 µM Ser/Thr 20 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:256 dilution of Development Reagent A is added.

PAK6
The 2X PAK6 / Ser/Thr 20 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.6 - 2.8 ng PAK6 and 2 µM Ser/Thr 20 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:256 dilution of Development Reagent A is added.

PAK7 (KIAA1264)
The 2X PAK7 (KIAA1264) / Ser/Thr 20 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.1 - 0.3 ng PAK7 (KIAA1264) and 2 µM Ser/Thr 20 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:256 dilution of Development Reagent A is added.

PASK
The 2X PASK / Ser/Thr 07 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 10.5 - 59.8 ng PASK and 2 µM Ser/Thr 07 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

PDGFRA (PDGFR alpha)
The 2X PDGFRA (PDGFR alpha) / Tyr 04 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 4 mM MnCl2, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 4.0 - 132.8 ng PDGFRA (PDGFR alpha) and 2 µM Tyr 04 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 2 mM MnCl2, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32 dilution of Development Reagent B is added.

PDGFRA D842V
The 2X PDGFRA D842V / Tyr 04 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 4 mM MnCl2, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 4.4 - 39.6 ng PDGFRA D842V and 2 µM Tyr 04 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 2 mM MnCl2, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32 dilution of Development Reagent B is added.

PDGFRA T674I
The 2X PDGFRA T674I / Tyr 04 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 4 mM MnCl2, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 14.6 - 58.4 ng PDGFRA T674I and 2 µM Tyr 04 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 2 mM MnCl2, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32 dilution of Development Reagent B is added.

PDGFRB (PDGFR beta)
The 2X PDGFRB (PDGFR beta) / Tyr 04 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 4 mM MnCl2, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 4.8 - 50.0 ng PDGFRB (PDGFR beta) and 2 µM Tyr 04 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 2 mM MnCl2, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32 dilution of Development Reagent B is added.

PHKG1
The 2X PHKG1 / Ser/Thr 07 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 4 mM CaCl2, 40 µg/ml Calmodulin, 0.02% NaN3. The final 10 µL Kinase Reaction consists of 6.3 - 117.8 ng PHKG1 and 2 µM Ser/Thr 07 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 500 µM EGTA, 2 mM CaCl2, 20 µg/ml Calmodulin, 0.01% NaN3. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

PHKG2
The 2X PHKG2 / Ser/Thr 07 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.3 - 9.2 ng PHKG2 and 2 µM Ser/Thr 07 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

PIM1
The 2X PIM1 / Ser/Thr 07 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.4 - 39.4 ng PIM1 and 2 µM Ser/Thr 07 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

PIM2
The 2X PIM2 / Ser/Thr 07 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2.5 - 20.0 ng PIM2 and 2 µM Ser/Thr 07 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

PKN1 (PRK1)
The 2X PKN1 (PRK1) / Ser/Thr 07 Peptide Mixture is prepared in 50 mM Tris pH 8.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA, 0.02% NaN3. The final 10 µL Kinase Reaction consists of 0.2 - 1.9 ng PKN1 (PRK1) and 2 µM Ser/Thr 07 Peptide in 50 mM Tris / HEPES pH 8.0, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA, 0.01% NaN3. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

PLK1
The 2X PLK1 / Ser/Thr 16 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2.8 - 25.3 ng PLK1 and 2 µM Ser/Thr 16 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:8 dilution of Development Reagent B is added.

PLK2
The 2X PLK2 / Ser/Thr 16 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 13.0 - 186.2 ng PLK2 and 2 µM Ser/Thr 16 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:8 dilution of Development Reagent B is added.

PLK3
The 2X PLK3 / Ser/Thr 16 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.8 - 9.0 ng PLK3 and 2 µM Ser/Thr 16 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:8 dilution of Development Reagent B is added.

PRKACA (PKA)
The 2X PRKACA (PKA) / Ser/Thr 01 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.0 - 0.2 ng PRKACA (PKA) and 2 µM Ser/Thr 01 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:2048 dilution of Development Reagent A is added.

PRKCA (PKC alpha)
The 2X PRKCA (PKC alpha) / Ser/Thr 07 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 4 mM CaCl2, 2X Novel Lipid Mix, 0.02% NaN3. The final 10 µL Kinase Reaction consists of 0.0 - 0.2 ng PRKCA (PKC alpha) and 2 µM Ser/Thr 07 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 500 µM EGTA, 2 mM CaCl2, 1X Novel Lipid Mix, 0.01% NaN3. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

PRKCB1 (PKC beta I)
The 2X PRKCB1 (PKC beta I) / Ser/Thr 07 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 4 mM CaCl2, 2X Novel Lipid Mix, 0.02% NaN3. The final 10 µL Kinase Reaction consists of 0.0 - 0.3 ng PRKCB1 (PKC beta I) and 2 µM Ser/Thr 07 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 500 µM EGTA, 2 mM CaCl2, 1X Novel Lipid Mix, 0.01% NaN3. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

PRKCB2 (PKC beta II)
The 2X PRKCB2 (PKC beta II) / Ser/Thr 07 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 4 mM CaCl2, 2X Novel Lipid Mix, 0.02% NaN3. The final 10 µL Kinase Reaction consists of 0.0 - 0.6 ng PRKCB2 (PKC beta II) and 2 µM Ser/Thr 07 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 500 µM EGTA, 2 mM CaCl2, 1X Novel Lipid Mix, 0.01% NaN3. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

PRKCD (PKC delta)
The 2X PRKCD (PKC delta) / Ser/Thr 07 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 4 mM CaCl2, 2X Novel Lipid Mix, 0.02% NaN3. The final 10 µL Kinase Reaction consists of 0.2 - 1.3 ng PRKCD (PKC delta) and 2 µM Ser/Thr 07 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 500 µM EGTA, 2 mM CaCl2, 1X Novel Lipid Mix, 0.01% NaN3. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

PRKCE (PKC epsilon)
The 2X PRKCE (PKC epsilon) / Ser/Thr 07 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 4 mM CaCl2, 2X Novel Lipid Mix, 0.02% NaN3. The final 10 µL Kinase Reaction consists of 0.1 - 0.7 ng PRKCE (PKC epsilon) and 2 µM Ser/Thr 07 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 500 µM EGTA, 2 mM CaCl2, 1X Novel Lipid Mix, 0.01% NaN3. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

PRKCG (PKC gamma)
The 2X PRKCG (PKC gamma) / Ser/Thr 07 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 4 mM CaCl2, 2X Novel Lipid Mix, 0.02% NaN3. The final 10 µL Kinase Reaction consists of 0.0 - 0.2 ng PRKCG (PKC gamma) and 2 µM Ser/Thr 07 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 500 µM EGTA, 2 mM CaCl2, 1X Novel Lipid Mix, 0.01% NaN3. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

PRKCH (PKC eta)
The 2X PRKCH (PKC eta) / Ser/Thr 07 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 4 mM CaCl2, 2X Novel Lipid Mix, 0.02% NaN3. The final 10 µL Kinase Reaction consists of 0.1 - 1.1 ng PRKCH (PKC eta) and 2 µM Ser/Thr 07 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 500 µM EGTA, 2 mM CaCl2, 1X Novel Lipid Mix, 0.01% NaN3. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

PRKCI (PKC iota)
The 2X PRKCI (PKC iota) / Ser/Thr 07 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 4 mM CaCl2, 2X Novel Lipid Mix, 0.02% NaN3. The final 10 µL Kinase Reaction consists of 0.8 - 5.0 ng PRKCI (PKC iota) and 2 µM Ser/Thr 07 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 500 µM EGTA, 2 mM CaCl2, 1X Novel Lipid Mix, 0.01% NaN3. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

PRKCN (PKD3)
The 2X PRKCN (PKD3) / Ser/Thr 17 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.3 - 5.8 ng PRKCN (PKD3) and 2 µM Ser/Thr 17 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

PRKCQ (PKC theta)
The 2X PRKCQ (PKC theta) / Ser/Thr 07 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 4 mM CaCl2, 2X Novel Lipid Mix, 0.02% NaN3. The final 10 µL Kinase Reaction consists of 0.0 - 0.4 ng PRKCQ (PKC theta) and 2 µM Ser/Thr 07 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 500 µM EGTA, 2 mM CaCl2, 1X Novel Lipid Mix, 0.01% NaN3. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

PRKCZ (PKC zeta)
The 2X PRKCZ (PKC zeta) / Ser/Thr 07 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 4 mM CaCl2, 2X Novel Lipid Mix, 0.02% NaN3. The final 10 µL Kinase Reaction consists of 1.0 - 8.0 ng PRKCZ (PKC zeta) and 2 µM Ser/Thr 07 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 500 µM EGTA, 2 mM CaCl2, 1X Novel Lipid Mix, 0.01% NaN3. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

PRKD1 (PKC mu)
The 2X PRKD1 (PKC mu) / Ser/Thr 17 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.6 - 4.8 ng PRKD1 (PKC mu) and 2 µM Ser/Thr 17 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

PRKD2 (PKD2)
The 2X PRKD2 (PKD2) / Ser/Thr 17 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.6 - 8.3 ng PRKD2 (PKD2) and 2 µM Ser/Thr 17 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

PRKG1
The 2X PRKG1 / Ser/Thr 01 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA, 20 µM cGMP. The final 10 µL Kinase Reaction consists of 0.1 - 0.8 ng PRKG1 and 2 µM Ser/Thr 01 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA, 10 µM cGMP. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:2048 dilution of Development Reagent A is added.

PRKG2 (PKG2)
The 2X PRKG2 (PKG2) / Ser/Thr 01 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA, 20 µM cGMP. The final 10 µL Kinase Reaction consists of 0.1 - 2.0 ng PRKG2 (PKG2) and 2 µM Ser/Thr 01 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA, 10 µM cGMP. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:2048 dilution of Development Reagent A is added.

PRKX
The 2X PRKX / Ser/Thr 01 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.3 - 5.1 ng PRKX and 2 µM Ser/Thr 01 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:2048 dilution of Development Reagent A is added.

PTK2 (FAK)
The 2X PTK2 (FAK) / Tyr 01 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2.7 - 40.1 ng PTK2 (FAK) and 2 µM Tyr 01 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:128 dilution of Development Reagent B is added.

PTK2B (FAK2)
The 2X PTK2B (FAK2) / Tyr 01 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MnCl2, 1 mM EGTA, 2 mM DTT, 0.02% NaN3. The final 10 µL Kinase Reaction consists of 7.1 - 69.7 ng PTK2B (FAK2) and 2 µM Tyr 01 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 5 mM MgCl2, 5 mM MnCl2, 1 mM EGTA, 1 mM DTT, 0.01% NaN3. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:128 dilution of Development Reagent B is added.

PTK6 (Brk)
The 2X PTK6 (Brk) / Tyr 01 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MnCl2, 1 mM EGTA, 2 mM DTT, 0.02% NaN3. The final 10 µL Kinase Reaction consists of 16.3 - 65.9 ng PTK6 (Brk) and 2 µM Tyr 01 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 5 mM MgCl2, 5 mM MnCl2, 1 mM EGTA, 1 mM DTT, 0.01% NaN3. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:128 dilution of Development Reagent B is added.

RET
The 2X RET / Tyr 02 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.5 - 4.6 ng RET and 2 µM Tyr 02 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

RET V804L
The 2X RET V804L / Tyr 02 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.5 - 5.0 ng RET V804L and 2 µM Tyr 02 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

RET Y791F
The 2X RET Y791F / Tyr 02 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.9 - 6.6 ng RET Y791F and 2 µM Tyr 02 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

ROCK1
The 2X ROCK1 / Ser/Thr 07 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.6 - 5.2 ng ROCK1 and 2 µM Ser/Thr 07 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

ROCK2
The 2X ROCK2 / Ser/Thr 13 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.9 - 6.1 ng ROCK2 and 2 µM Ser/Thr 13 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:512 dilution of Development Reagent A is added.

ROS1
The 2X ROS1 / Tyr 01 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 3.6 - 23.9 ng ROS1 and 2 µM Tyr 01 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:128 dilution of Development Reagent B is added.

RPS6KA1 (RSK1)
The 2X RPS6KA1 (RSK1) / Ser/Thr 06 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.8 - 7.1 ng RPS6KA1 (RSK1) and 2 µM Ser/Thr 06 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:2048 dilution of Development Reagent A is added.

RPS6KA2 (RSK3)
The 2X RPS6KA2 (RSK3) / Ser/Thr 06 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2.3 - 15.6 ng RPS6KA2 (RSK3) and 2 µM Ser/Thr 06 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:2048 dilution of Development Reagent A is added.

RPS6KA3 (RSK2)
The 2X RPS6KA3 (RSK2) / Ser/Thr 06 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.3 - 2.7 ng RPS6KA3 (RSK2) and 2 µM Ser/Thr 06 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:2048 dilution of Development Reagent A is added.

RPS6KA4 (MSK2)
The 2X RPS6KA4 (MSK2) / Ser/Thr 01 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 12.4 - 126.1 ng RPS6KA4 (MSK2) and 2 µM Ser/Thr 01 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:2048 dilution of Development Reagent A is added.

RPS6KA5 (MSK1)
The 2X RPS6KA5 (MSK1) / Ser/Thr 01 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.0 - 11.0 ng RPS6KA5 (MSK1) and 2 µM Ser/Thr 01 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:2048 dilution of Development Reagent A is added.

RPS6KA6 (RSK4)
The 2X RPS6KA6 (RSK4) / Ser/Thr 20 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.1 - 2.0 ng RPS6KA6 (RSK4) and 2 µM Ser/Thr 20 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:256 dilution of Development Reagent A is added.

RPS6KB1 (p70S6K)
The 2X RPS6KB1 (p70S6K) / Ser/Thr 07 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 6.3 - 51.3 ng RPS6KB1 (p70S6K) and 2 µM Ser/Thr 07 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

SGK (SGK1)
The 2X SGK (SGK1) / Ser/Thr 06 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.1 - 1.0 ng SGK (SGK1) and 2 µM Ser/Thr 06 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:2048 dilution of Development Reagent A is added.

SGK2
The 2X SGK2 / Ser/Thr 06 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.2 - 3.0 ng SGK2 and 2 µM Ser/Thr 06 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:2048 dilution of Development Reagent A is added.

SGKL (SGK3)
The 2X SGKL (SGK3) / Ser/Thr 06 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.1 - 1.8 ng SGKL (SGK3) and 2 µM Ser/Thr 06 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:2048 dilution of Development Reagent A is added.

SRC
The 2X SRC / Tyr 02 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 3.4 - 36.0 ng SRC and 2 µM Tyr 02 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

SRC N1
The 2X SRC N1 / Tyr 02 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.9 - 15.5 ng SRC N1 and 2 µM Tyr 02 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

SRMS (Srm)
The 2X SRMS (Srm) / Tyr 01 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 13.3 - 67.7 ng SRMS (Srm) and 2 µM Tyr 01 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:128 dilution of Development Reagent B is added.

SRPK1
The 2X SRPK1 / Ser/Thr 18 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 7.5 - 79.3 ng SRPK1 and 2 µM Ser/Thr 18 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

SRPK2
The 2X SRPK2 / Ser/Thr 07 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 10.6 - 123.1 ng SRPK2 and 2 µM Ser/Thr 07 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

STK22B (TSSK2)
The 2X STK22B (TSSK2) / Ser/Thr 04 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 5.6 - 37.5 ng STK22B (TSSK2) and 2 µM Ser/Thr 04 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

STK22D (TSSK1)
The 2X STK22D (TSSK1) / Ser/Thr 04 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.5 - 11.4 ng STK22D (TSSK1) and 2 µM Ser/Thr 04 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

STK23 (MSSK1)
The 2X STK23 (MSSK1) / Ser/Thr 18 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 6.9 - 30.6 ng STK23 (MSSK1) and 2 µM Ser/Thr 18 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

STK24 (MST3)
The 2X STK24 (MST3) / Ser/Thr 07 Peptide Mixture is prepared in 50 mM Tris pH 8.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA, 0.02% NaN3. The final 10 µL Kinase Reaction consists of 14.7 - 84.0 ng STK24 (MST3) and 2 µM Ser/Thr 07 Peptide in 50 mM Tris / HEPES pH 8.0, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA, 0.01% NaN3. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

STK25 (YSK1)
The 2X STK25 (YSK1) / Ser/Thr 07 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 3.1 - 56.8 ng STK25 (YSK1) and 2 µM Ser/Thr 07 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

STK3 (MST2)
The 2X STK3 (MST2) / Ser/Thr 07 Peptide Mixture is prepared in 50 mM HEPES pH 6.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA, 0.02% NaN3. The final 10 µL Kinase Reaction consists of 5.7 - 150.0 ng STK3 (MST2) and 2 µM Ser/Thr 07 Peptide in 50 mM HEPES pH 7.0, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA, 0.01% NaN3. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

STK4 (MST1)
The 2X STK4 (MST1) / Ser/Thr 07 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 8.0 - 200.0 ng STK4 (MST1) and 2 µM Ser/Thr 07 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

STK6 (Aurora A)
The 2X STK6 (Aurora A) / Ser/Thr 01 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.9 - 6.2 ng STK6 (Aurora A) and 2 µM Ser/Thr 01 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:2048 dilution of Development Reagent A is added.

SYK
The 2X SYK / Tyr 02 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.8 - 6.4 ng SYK and 2 µM Tyr 02 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

TAOK2 (TAO1)
The 2X TAOK2 (TAO1) / Ser/Thr 07 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 8.1 - 70.4 ng TAOK2 (TAO1) and 2 µM Ser/Thr 07 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

TBK1
The 2X TBK1 / Ser/Thr 05 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.6 - 9.2 ng TBK1 and 2 µM Ser/Thr 05 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:128 dilution of Development Reagent B is added.

TEK (Tie2)
The 2X TEK (Tie2) / Tyr 05 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 4 mM MnCl2, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 0.5 - 5.8 ng TEK (Tie2) and 2 µM Tyr 05 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 2 mM MnCl2, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

TYRO3 (RSE)
The 2X TYRO3 (RSE) / Tyr 02 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2.8 - 40.8 ng TYRO3 (RSE) and 2 µM Tyr 02 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

YES1
The 2X YES1 / Tyr 02 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.5 - 18.0 ng YES1 and 2 µM Tyr 02 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

ZAP70
The 2X ZAP70 / Tyr 07 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MnCl2, 1 mM EGTA, 2 mM DTT, 0.02% NaN3. The final 10 µL Kinase Reaction consists of 8.8 - 100.0 ng ZAP70 and 2 µM Tyr 07 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 5 mM MgCl2, 5 mM MnCl2, 1 mM EGTA, 1 mM DTT, 0.01% NaN3. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:16 dilution of Development Reagent B is added.

Kinase-Specific Assay Conditions – Cascade Format

BRAF
The 2X BRAF / inactive MAP2K1 (MEK1) / inactive MAPK1 (ERK2) / Ser/Thr 03 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.02 – 0.07 ng BRAF, 10 ng inactive MAP2K1 (MEK1), 100 ng inactive MAPK1 (ERK2), and 2 µM Ser/Thr 03 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

BRAF V599E
The 2X BRAF V599E / inactive MAP2K1 (MEK1) / inactive MAPK1 (ERK2) / Ser/Thr 03 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.002 – 0.007 ng BRAF V599E, 10 ng inactive MAP2K1 (MEK1), 100 ng inactive MAPK1 (ERK2), and 2 µM Ser/Thr 03 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

MAP2K1 (MEK1)
The 2X MAP2K1 (MEK1) / inactive MAPK1 (ERK2) / Ser/Thr 03 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.0 - 4.0 ng MAP2K1 (MEK1), 105 ng inactive MAPK1 (ERK2), and 2 µM Ser/Thr 03 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

MAP2K2 (MEK2)
The 2X MAP2K2 (MEK2) / inactive MAPK1 (ERK2) / Ser/Thr 03 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.0 - 4.0 ng MAP2K2 (MEK2), 105 ng inactive MAPK1 (ERK2), and 2 µM Ser/Thr 03 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

MAP2K6 (MKK6)
The 2X MAP2K6 (MKK6) / inactive MAPK12 (p38 gamma) / Ser/Thr 03 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 9.76 - 39.02 ng MAP2K6 (MKK6), 100 ng inactive MAPK1K (p38 gamma), and 2 µM Ser/Thr 03 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

MAP3K8 (COT)
The 2X MAP3K8 (COT) / inactive MAP2K1 (MEK1) / inactive MAPK1 (ERK2) / Ser/Thr 03 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.25 – 1.00 ng MAP3K8 (COT), 10 ng inactive MAP2K1 (MEK1), 100 ng inactive MAPK1 (ERK2), and 2 µM Ser/Thr 03 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

MAPK14 (p38 alpha)
The 2X MAPK14 (p38 alpha) / inactive MAPKAPK2 / Ser/Thr 04 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.01 - 0.02 ng MAPK14 (p38 alpha), 5 ng inactive MAPKAPK2, and 2 µM Ser/Thr 04 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

PDK1
The 2X PDK1 / inactive AKT2 (PKB beta) / Ser/Thr 06 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 3.16 - 12.62 ng PDK1, 150 ng inactive AKT2 (PKB beta), and 2 µM Ser/Thr 06 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:2048 dilution of Development Reagent A is added.

RAF1 (cRAF)
The 2X RAF1 (cRAF) / inactive MAP2K1 (MEK1) / inactive MAPK1 (ERK2) / Ser/Thr 03 Peptide Mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.001 – 0.005 ng RAF1 (cRAF), 10 ng inactive MAP2K1 (MEK1), 100 ng inactive MAPK1 (ERK2), and 2 µM Ser/Thr 03 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

TABLE OF KINASE ATP Km BINS AND INHIBITOR VALIDATION

The table below provides specifications and data around each kinase. The representative IC_{50} value with a known inhibitor for each kinase was determined at the ATP bin nearest to the ATP $K_{m\ app}$, unless indicated with an asterisk (*) in which case the IC_{50} value was determined at 100 µM ATP.

Kinase	Z'-LYTE Substrate	ATP $K_{m\ app}$ (µM)	ATP Bin (µM)	Inhibitor	IC50 (nM)
ABL1	Tyr 02	12	10	Tyrphostin AG1478	430
ABL1 E255K	Tyr 02	4	5	Staurosporine	100
ABL1 G250E	Tyr 02	5	5	Staurosporine	53
ABL1 T315I	Tyr 02	3	5	Staurosporine	18
ABL1 Y253F	Tyr 02	12	10	Staurosporine	79
ABL2 (Arg)	Tyr 02	30	25	Tyrphostin AG1478	1000
ACVR1B (ALK4)	Ser/Thr 16	2	5	SB 431542	790
ADRBK1 (GRK2)	Ser/Thr 16	13	10	Staurosporine	2200
ADRBK2 (GRK3)	Ser/Thr 16	12	10	Staurosporine	2400
AKT1 (PKB alpha)	Ser/Thr 06	75	75	Staurosporine	37
AKT2 (PKB beta)	Ser/Thr 06	200	200	Staurosporine	120
AKT3 (PKB gamma)	Ser/Thr 06	100	100	Staurosporine	25
ALK	Tyr 01	30	25	Staurosporine	8.4
AURKB (Aurora B)	Ser/Thr 01	64	75	Staurosporine	8.7
AURKC (Aurora C)	Ser/Thr 19	26	25	Staurosporine	8.7
BLK	Tyr 01	30	25	Tyrphostin AG1478	1900
BMX	Tyr 01	107	100	Tyrphostin AG1478	37000
BRAF	Ser/Thr 03	Cascade	100	Staurosporine	80 *
BRAF V599E	Ser/Thr 03	Cascade	100	Staurosporine	85 *
BRSK1 (SAD1)	Ser/Thr 21	32	25	Staurosporine	2.4
BTK	Tyr 01	36	25	Tyrphostin AG1478	6000
CAMK1D (CaMKI delta)	Ser/Thr 10	16	10	Staurosporine	26
CAMK2A (CaMKII alpha)	Ser/Thr 04	10	10	Staurosporine	2.9
CAMK2B (CaMKII beta)	Ser/Thr 17	76	75	Staurosporine	2.1
CAMK2D (CaMKII delta)	Ser/Thr 04	6	5	Staurosporine	0.7
CAMK4 (CaMKIV)	Ser/Thr 13	18	25	Staurosporine	250
CDC42 BPA (MRCKA)	Ser/Thr 13	1	5	Staurosporine	31
CDC42 BPB (MRCKB)	Ser/Thr 13	1	5	Staurosporine	15
CDK1/cyclin B	Ser/Thr 18	34	25	Staurosporine	10
CDK2/cyclin A	Ser/Thr 12	31	25	Staurosporine	5.9
CDK5/p35	Ser/Thr 12	8	10	Staurosporine	9.2
CHEK1 (CHK1)	Ser/Thr 07	45	50	Staurosporine	14
CHEK2 (CHK2)	Ser/Thr 07	84	75	Staurosporine	26
CLK1	Ser/Thr 09	24	25	Staurosporine	140
CLK2	Ser/Thr 06	30	25	Staurosporine	19
CLK3	Ser/Thr 18	128	150	Staurosporine	2300
CSF1R (FMS)	Tyr 01	450	500	Tyrphostin AG1478	6000
CSK	Tyr 02	15	10	Staurosporine	97
CSNK1A1 (CK1 alpha 1)	Ser/Thr 11	2	5	TBB	18000
CSNK1D (CK1 delta)	Ser/Thr 11	4	5	TBB	3700
CSNK1E (CK1 epsilon)	Ser/Thr 11	2	5	TBB	4800
CSNK1G1 (CK1 gamma 1)	Ser/Thr 05	2	5	Chetomin	110
CSNK1G2 (CK1 gamma 2)	Ser/Thr 05	5	5	TBB	27000
CSNK1G3 (CK1 gamma 3)	Ser/Thr 05	4	5	TBB	70000
CSNK2A1 (CK2 alpha 1)	Ser/Thr 11	4	5	TBB	3000
CSNK2A2 (CK2 alpha 2)	Ser/Thr 11	46	50	TBB	2400
DAPK3 (ZIPK)	Ser/Thr 13	3	5	Staurosporine	45
DCAMKL2 (DCK2)	Ser/Thr 17	150	150	Staurosporine	78
DYRK1A	Ser/Thr 18	100	100	Staurosporine	32
DYRK3	Ser/Thr 09	5	5	Staurosporine	190
DYRK4	Ser/Thr 09	2	5	TBB	13000
EGFR (ErbB1)	Tyr 04	12	10	Staurosporine	150
EGFR L858R (ErbB1 L858R)	Tyr 04	44	50	Staurosporine	150
EGFR L861Q (ErbB1 L861Q)	Tyr 04	13	10	Staurosporine	88

Kinase	Z'-LYTE Substrate	ATP $K_{m\,app}$ (µM)	ATP Bin (µM)	Inhibitor	IC50 (nM)
EPHA1	Tyr 02	19	25	Staurosporine	83
EPHA2	Tyr 01	65	75	Staurosporine	290
EPHA3	Tyr 01	300	300	Tyrphostin AG1478	15000
EPHA4	Tyr 02	106	100	Staurosporine	100
EPHA5	Tyr 01	129	150	Staurosporine	110
EPHA8	Tyr 02	123	100	Staurosporine	180
EPHB1	Tyr 02	61	50	Staurosporine	170
EPHB2	Tyr 02	66	75	Staurosporine	220
EPHB3	Tyr 02	70	75	PP2	760
EPHB4	Tyr 01	115	100	Tyrphostin AG1478	1600
ERBB2 (HER2)	Tyr 06	14	10	Tyrphostin AG1478	210
ERBB4 (HER4)	Tyr 01	5	5	Tyrphostin AG1478	140
FER	Tyr 05	19	25	Staurosporine	4.8
FES (FPS)	Tyr 01	30	25	Staurosporine	4.6
FGFR1	Tyr 04	20	25	Staurosporine	18
FGFR2	Tyr 04	1	5	Staurosporine	5.3
FGFR3	Tyr 04	80	75	Staurosporine	67
FGFR3 K650E	Tyr 04	6	5	Staurosporine	8.5
FGFR4	Tyr 04	162	150	Staurosporine	270
FGR	Tyr 02	10	10	Tyrphostin AG1478	920
FLT1 (VEGFR1)	Tyr 04	158	150	Staurosporine	37
FLT3	Tyr 02	470	500	Tyrphostin AG1478	2900
FLT3 D835Y	Tyr 02	19	25	Staurosporine	0.5
FLT4 (VEGFR3)	Tyr 04	4	5	Staurosporine	5.8
FRK (PTK5)	Tyr 01	52	50	Staurosporine	17
FYN	Tyr 02	85	75	Tyrphostin AG1478	6800
GRK4	Ser/Thr 16	12	10	Staurosporine	170
GRK5	Ser/Thr 16	3	5	Staurosporine	260
GRK6	Ser/Thr 16	12	10	Staurosporine	120
GRK7	Ser/Thr 16	11	10	Staurosporine	8.2
GSK3A (GSK3 alpha)	Ser/Thr 09	10	10	Staurosporine	27
GSK3B (GSK3 beta)	Ser/Thr 09	7	10	Staurosporine	20
HCK	Tyr 02	59	50	Tyrphostin AG1478	1200
HIPK1 (Myak)	Ser/Thr 09	5	5	TBB	4500
HIPK4	Ser/Thr 18	39	50	Staurosporine	800
IGF1R	Tyr 01	140	150	Staurosporine	230
IKBKB (IKK beta)	Ser/Thr 05	5	5	Staurosporine	450
INSR	Tyr 01	20	25	Staurosporine	220
INSRR (IRR)	Tyr 04	55	50	Staurosporine	300
IRAK4	Ser/Thr 07	34	25	Staurosporine	33
ITK	Tyr 01	6	5	Staurosporine	49
JAK2	Tyr 06	2	5	Staurosporine	3.5
JAK2 JH1 JH2	Tyr 06	46	50	Staurosporine	1.4
JAK3	Tyr 04	< 5	5	Staurosporine	7.2
KDR (VEGFR2)	Tyr 01	78	75	Staurosporine	7.4
KIT	Tyr 06	284	300	Staurosporine	490
KIT T670I	Tyr 06	220	200	Staurosporine	200
LCK	Tyr 02	45	50	Tyrphostin AG1478	1500
LYN A	Tyr 02	25	10	Tyrphostin AG1478	210
LYN B	Tyr 02	25	10	Tyrphostin AG1478	240
MAP2K1 (MEK1)	Ser/Thr 03	Cascade	100	Staurosporine	4.9 *
MAP2K2 (MEK2)	Ser/Thr 03	Cascade	100	Staurosporine	10 *
MAP2K6 (MKK6)	Ser/Thr 03	Cascade	100	Staurosporine	17 *
MAP3K8 (COT)	Ser/Thr 03	Cascade	100	Staurosporine	41 *

Kinase	Z'-LYTE Substrate	ATP $K_{m\,app}$ (µM)	ATP Bin (µM)	Inhibitor	IC50 (nM)
MAP3K9 (MLK1)	Ser/Thr 07	73	75	Staurosporine	5.8
MAP4K2 (GCK)	Ser/Thr 07	109	100	Staurosporine	3.5
MAP4K4 (HGK)	Ser/Thr 07	13	10	Staurosporine	2.6
MAP4K5 (KHS1)	Ser/Thr 07	55	50	Staurosporine	2.3
MAPK1 (ERK2)	Ser/Thr 03	100	100	Staurosporine	2500
MAPK11 (p38 beta)	Ser/Thr 15	39	50	PP2	3500
MAPK12 (p38 gamma)	Ser/Thr 03	16	10	Staurosporine	980
MAPK13 (p38 delta)	Ser/Thr 03	13	10	Staurosporine	410
MAPK14 (p38 alpha)	Ser/Thr 04	Cascade	100	Staurosporine	7900 *
MAPK3 (ERK1)	Ser/Thr 03	45	50	Staurosporine	3300
MAPKAPK2	Ser/Thr 04	3	5	Staurosporine	720
MAPKAPK3	Ser/Thr 04	200	200	Staurosporine	23000
MAPKAPK5 (PRAK)	Ser/Thr 04	14	10	Staurosporine	870
MARK1 (MARK)	Ser/Thr 21	7	5	Staurosporine	19
MARK2	Ser/Thr 21	12	10	Staurosporine	16
MATK (HYL)	Tyr 01	350	300	Staurosporine	1000
MERTK (cMER)	Tyr 02	15	10	Staurosporine	75
MET (cMet)	Tyr 06	64	50	Staurosporine	160
MET M1250T	Tyr 06	14	10	Staurosporine	310
MINK1	Ser/Thr 07	27	25	Staurosporine	2.3
MST1R (RON)	Tyr 06	11	10	Staurosporine	74
MST4	Ser/Thr 07	28	25	Staurosporine	7.5
MUSK	Tyr 04	50	50	Staurosporine	9.3
MYLK2 (skMLCK)	Ser/Thr 13	310	300	Staurosporine	240
NEK1	Ser/Thr 07	119	100	Staurosporine	120
NEK2	Ser/Thr 07	150	150	Staurosporine	5900
NEK4	Ser/Thr 17	56	50	Staurosporine	340
NTRK1 (TRKA)	Tyr 01	425	400	Staurosporine	3.2
NTRK2 (TRKB)	Tyr 01	22	25	Staurosporine	0.9
NTRK3 (TRKC)	Tyr 01	54	50	Staurosporine	2.5
PAK2 (PAK65)	Ser/Thr 20	390	400	Staurosporine	77
PAK3	Ser/Thr 20	132	150	Staurosporine	44
PAK4	Ser/Thr 20	3	5	Staurosporine	9.7
PAK6	Ser/Thr 20	8	10	Staurosporine	7
PAK7 (KIAA1264)	Ser/Thr 20	4	5	Staurosporine	26
PASK	Ser/Thr 07	53	50	Staurosporine	96
PDGFRA (PDGFR alpha)	Tyr 04	67	75	Staurosporine	8.4
PDGFRA D842V	Tyr 04	5	5	Staurosporine	14
PDGFRA T674I	Tyr 04	93	100	Staurosporine	8.1
PDGFRB (PDGFR beta)	Tyr 04	100	100	Staurosporine	6.4
PDK1	Ser/Thr 06	Cascade	100	Staurosporine	8.3 *
PHKG1	Ser/Thr 07	63	75	Staurosporine	1.1
PHKG2	Ser/Thr 07	10	10	Staurosporine	3.3
PIM1	Ser/Thr 07	407	400	TBB	2000
PIM2	Ser/Thr 07	3	5	Staurosporine	65
PKN1 (PRK1)	Ser/Thr 07	40	50	Staurosporine	1.9
PLK1	Ser/Thr 16	13	10	Staurosporine	820
PLK2	Ser/Thr 16	30	25	Staurosporine	630
PLK3	Ser/Thr 16	48	50	TBB	3600
PRKACA (PKA)	Ser/Thr 01	4	5	Staurosporine	3.4
PRKCA (PKC alpha)	Ser/Thr 07	37	25	Staurosporine	2.2
PRKCB1 (PKC beta I)	Ser/Thr 07	250	200	Staurosporine	3.6
PRKCB2 (PKC beta II)	Ser/Thr 07	225	200	Staurosporine	2.2
PRKCD (PKC delta)	Ser/Thr 07	30	25	Staurosporine	1.6

Kinase	Z'-LYTE Substrate	ATP $K_{m\,app}$ (µM)	ATP Bin (µM)	Inhibitor	IC50 (nM)
PRKCE (PKC epsilon)	Ser/Thr 07	35	25	Staurosporine	2.4
PRKCG (PKC gamma)	Ser/Thr 07	25	25	Staurosporine	1.9
PRKCH (PKC eta)	Ser/Thr 07	35	25	Staurosporine	5.1
PRKCI (PKC iota)	Ser/Thr 07	25	25	Staurosporine	410
PRKCN (PKD3)	Ser/Thr 17	26	25	Staurosporine	13
PRKCQ (PKC theta)	Ser/Thr 07	100	100	Staurosporine	4.1
PRKCZ (PKC zeta)	Ser/Thr 07	4	5	Staurosporine	1100
PRKD1 (PKC mu)	Ser/Thr 17	15	10	Staurosporine	5.9
PRKD2 (PKD2)	Ser/Thr 17	27	25	Staurosporine	5
PRKG1	Ser/Thr 01	20	25	Staurosporine	9.8
PRKG2 (PKG2)	Ser/Thr 01	177	150	Staurosporine	1.8
PRKX	Ser/Thr 01	17	10	Staurosporine	3.9
PTK2 (FAK)	Tyr 01	46	50	Staurosporine	28
PTK2B (FAK2)	Tyr 01	5	5	Staurosporine	34
PTK6 (Brk)	Tyr 01	82	75	Staurosporine	1200
RAF1 (cRAF)	Ser/Thr 03	Cascade	100	Staurosporine	35 *
RET	Tyr 02	11	10	Staurosporine	5.1
RET V804L	Tyr 02	5	5	Staurosporine	3.3
RET Y791F	Tyr 02	12	10	Staurosporine	4.4
ROCK1	Ser/Thr 07	3	5	Staurosporine	7.2
ROCK2	Ser/Thr 13	40	50	Staurosporine	5.4
ROS1	Tyr 01	61	50	Staurosporine	5.4
RPS6KA1 (RSK1)	Ser/Thr 06	7	5	Staurosporine	1.6
RPS6KA2 (RSK3)	Ser/Thr 06	11	10	Staurosporine	1.1
RPS6KA3 (RSK2)	Ser/Thr 06	18	10	Staurosporine	0.8
RPS6KA4 (MSK2)	Ser/Thr 01	14	10	Staurosporine	3.9
RPS6KA5 (MSK1)	Ser/Thr 01	39	50	Staurosporine	5.4
RPS6KA6 (RSK4)	Ser/Thr 20	30	25	Staurosporine	0.7
RPS6KB1 (p70S6K)	Ser/Thr 07	17	10	Staurosporine	6.9
SGK (SGK1)	Ser/Thr 06	36	25	Staurosporine	20
SGK2	Ser/Thr 06	50	50	Staurosporine	85
SGKL (SGK3)	Ser/Thr 06	26	25	Staurosporine	19
SRC	Tyr 02	50	50	Tyrphostin AG1478	3200
SRC N1	Tyr 02	50	50	Tyrphostin AG1478	2000
SRMS (Srm)	Tyr 01	127	150	Staurosporine	320
SRPK1	Ser/Thr 18	32	25	Staurosporine	560
SRPK2	Ser/Thr 07	18	25	Staurosporine	2200
STK22B (TSSK2)	Ser/Thr 04	3	5	Staurosporine	20
STK22D (TSSK1)	Ser/Thr 04	9	10	Staurosporine	0.9
STK23 (MSSK1)	Ser/Thr 18	69	75	Staurosporine	3500
STK24 (MST3)	Ser/Thr 07	56	50	Staurosporine	10
STK25 (YSK1)	Ser/Thr 07	83	75	Staurosporine	7.2
STK3 (MST2)	Ser/Thr 07	50	50	Staurosporine	6.5
STK4 (MST1)	Ser/Thr 07	49	50	Staurosporine	11
STK6 (Aurora A)	Ser/Thr 01	10	10	Staurosporine	3.9
SYK	Tyr 02	25	25	Staurosporine	0.7
TAOK2 (TAO1)	Ser/Thr 07	322	300	Staurosporine	36
TBK1	Ser/Thr 05	31	25	Staurosporine	1.7
TEK (Tie2)	Tyr 05	17	10	Staurosporine	120
TYRO3 (RSE)	Tyr 02	28	25	Staurosporine	11
YES1	Tyr 02	155	150	Tyrphostin AG1478	2600
ZAP70	Tyr 07	2	5	Staurosporine	430