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**Optimization of the GeneBLAzer® C5AR1-Gα15-NFAT-*bla* CHO-K1 Cell Line**

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**GeneBLAzer® C5AR1-Gα15-NFAT-*bla* CHO-K1 Cells****GeneBLAzer® C5AR1-Gα15 CHO-K1 DA Cells**

Catalog Numbers – K1746, K1544

**Cell Line Description**

GeneBLAzer® C5AR1-Gα15 CHO-K1 DA (Division Arrested) cells and GeneBLAzer® C5AR1-Gα15-NFAT-*bla* CHO-K1 cells contain the human Complement Component 5a Receptor 1 (C5AR1) (Accession # [NM\\_000710](#)) stably integrated into the GeneBLAzer® Gα15-NFAT-*bla* CHO-K1 cell line. GeneBLAzer® Gα15-NFAT-*bla* CHO-K1 (Cat. No. K1539) contains a beta-lactamase reporter gene under control of a NFAT response element and a promiscuous G Protein, Gα15, stably integrated into CHO-K1 cells.

DA cells are irreversibly division arrested using a low-dose treatment of Mitomycin-C, and have no apparent toxicity or change in cellular signal transduction. Both GeneBLAzer® C5AR1-Gα15 CHO-K1 DA cells and GeneBLAzer® C5AR1-Gα15-NFAT-*bla* CHO-K1 cells are functionally validated for Z'-factor and EC<sub>50</sub> concentrations using C5a. In addition, GeneBLAzer® C5AR1-Gα15-NFAT-*bla* CHO-K1 cells have been tested for assay performance under variable conditions, including DMSO concentration, cell number, stimulation time, and substrate loading time. Additional testing data using alternate stimuli are also available.

**Target Description**

The complement system is typically involved in an immune response to infectious organisms, tissue damage, or other substances not recognized as 'self'. Once the cascade is initiated, many fragments of various complement proteins are generated. Some of these proteins combine to form a membrane attack complex which can perforate the cell walls of invading cells, while others bind receptors on the surface of cells (reviewed in 1). C5a is one of the many peptides formed during the cascade. It is known as an anaphylatoxin and is known to stimulate the release of histamine from mast cells (2), and attract neutrophils (3,4) and macrophages (5).

C5a binds two G-coupled protein receptors, C5AR1 (6,7) and C5L2 (8). Studies have found that C5L2 is unable to couple to G-proteins and may be a decoy receptor. C5AR1 is Gαi coupled and requires the promiscuous G-protein Gα15 to signal through the NFAT-*bla* response element in this cell line. C5AR1 is expressed in the cells of the immune system as well as the kidney, liver, lungs, and some nerve cells (reviewed in 1). Antagonists of C5AR1 could have therapeutic benefits for shock patients as well as a variety of inflammatory diseases including rheumatoid arthritis, lupus, and tissue rejection. Agonists of this receptor may help boost immune response (reviewed in 1).

## Validation Results

Performance of this assay was evaluated under various conditions in 384-well format using LiveBLAzer™-FRET B/G Substrate.

### 1. C5a dose response under optimized conditions (n=3)

	<u>DA cells</u>	<u>Dividing Cells</u>
EC <sub>50</sub>	33 ng/mL	27 ng/mL
Z'-factor	0.82	0.88

Recommended cell no.	= 10K cells/well
Recommended [DMSO]	= up to 1%
Recommended Stim. Time	= 5 hours
Max. [Stimulation]	= 2 mg/mL

### 2. Alternate agonist dose response

C5a (65-74) EC<sub>50</sub> = 2.92 μM

### 3. Antagonist dose response

None available

### 4. Agonist 2<sup>nd</sup> messenger dose response

C5a EC<sub>50</sub> = 11 ng/mL

## Assay Performance with Variable Conditions

### 5. Assay performance with variable cell number

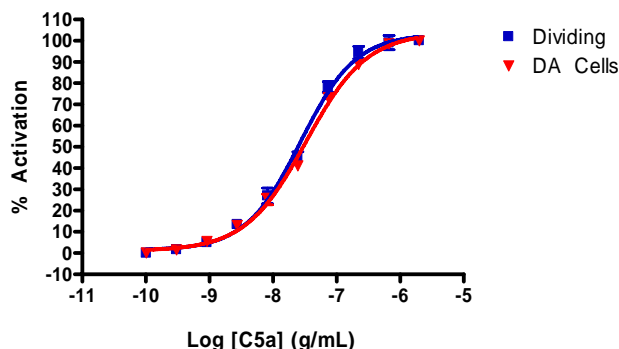
### 6. Assay performance with variable stimulation time

### 7. Assay performance with variable substrate loading time

### 8. Assay performance with variable DMSO concentration

## Primary Agonist Dose Response

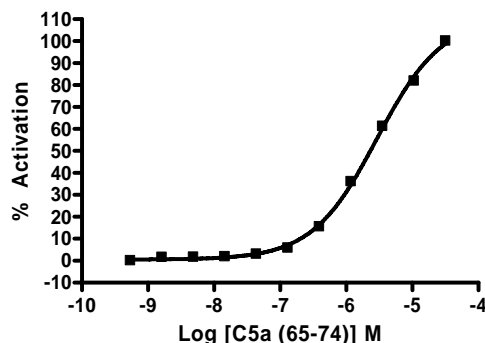
**Figure 1** —C5a dose response under optimized conditions



GeneBLAzer® C5AR1-Gα15-NFAT-*bla* CHO-K1 cells and GeneBLAzer® C5AR1-Gα15-NFAT-*bla* CHO-K1 DA Cells (10,000 cells/well) were assayed on three separate days. Cells were plated the day before the assay in a 384-well format and stimulated with C5a (R & D Systems 2037-C5) over the indicated concentration range in the presence of 0.5% DMSO for 5 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate for 2 hours. Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and the % Activation plotted against the indicated concentrations of C5a (n=6 for each data point).

## Alternate Agonist Dose Response

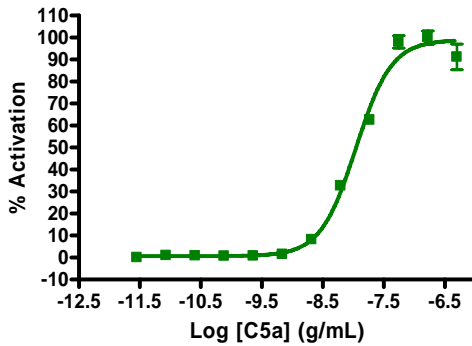
**Figure 2** —C5a (65-74) dose response under optimized conditions



GeneBLAzer® C5AR1-Gα15-NFAT-*bla* CHO-K1 cells (10,000 cells/well) were plated the day before the assay in a 384-well format and stimulated with C5a (65-74) (Bachem #H-3462.0001) over the indicated concentration range in the presence of 0.5% DMSO for 5 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate for 2 hours. Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and the % Activation plotted against the indicated concentrations of C5a (65-74)(n=16) for each data point).

### Agonist 2<sup>nd</sup> Messenger Response

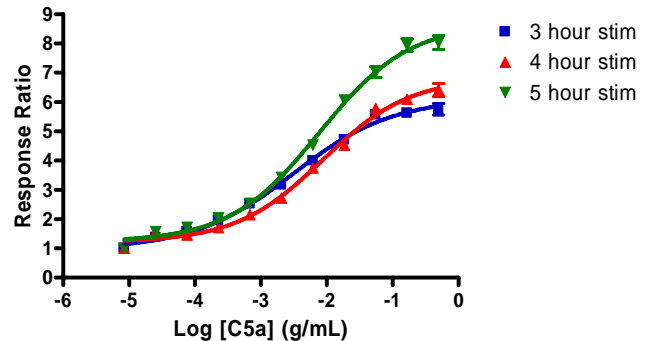
**Figure 3— GeneBLAzer® C5AR1-Gα15-NFAT-*bla* CHO-K1 2<sup>nd</sup> messenger dose response to C5a under optimized conditions**



GeneBLAzer® C5AR1-Gα15-NFAT-*bla* CHO-K1 cells were loaded with Fluo4-AM and tested for a response to C5a.

### Assay Performance with Variable Stimulation Time

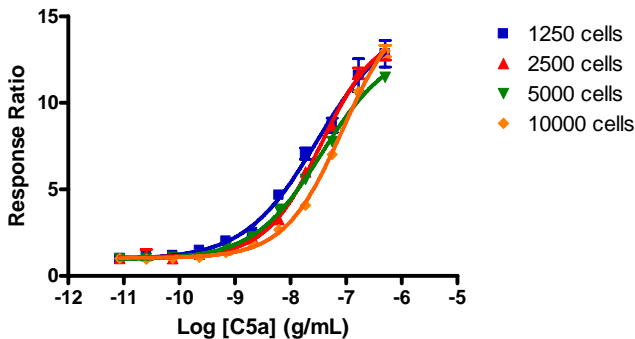
**Figure 5 –C5a dose response with 3, 4 and 5 hr stimulation times**



GeneBLAzer® C5AR1-Gα15-NFAT-*bla* CHO-K1 cells (10,000 cells/well) were plated the day before the assay in a 384-well assay plate. C5a (R & D Systems 2037-C5) was then added to the plate over the indicated concentration range for 3, 4, or 5 hrs in 0.5% DMSO and then loaded for 2 hours with LiveBLAzer™-FRET B/G Substrate. Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and the Response Ratios plotted against the indicated concentrations of C5a (n=8 for each data point).

### Assay Performance with Variable Cell Number

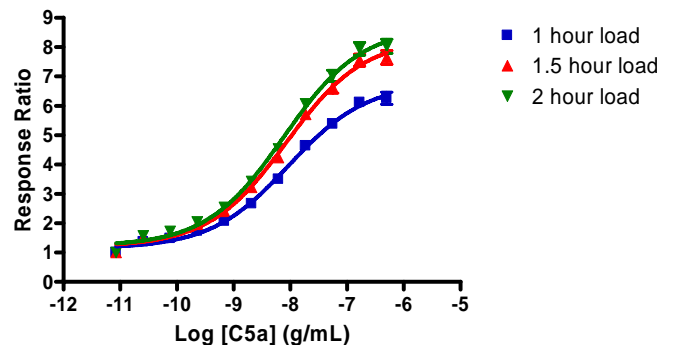
**Figure 4— C5a Dose response using 1.25, 2.5, 5, and 10K cells/well**



GeneBLAzer® C5AR1-Gα15-NFAT-*bla* CHO-K1 cells were plated the day before the assay at 1,250 2,500 or 5,000 and 10,000 cells/well in a 384-well format. On the day of the assay, cells were stimulated C5a (R & D Systems 2037-C5) in the presence of 0.5% DMSO for 5 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate for 2 hours. Fluorescence emission values at 460 nm and 530 nm for the various cell numbers were obtained using a standard fluorescence plate reader and the Response Ratios plotted for each cell number against the indicated concentrations of C5a (n=8 for each data point).

### Assay Performance with Variable Substrate Loading Times

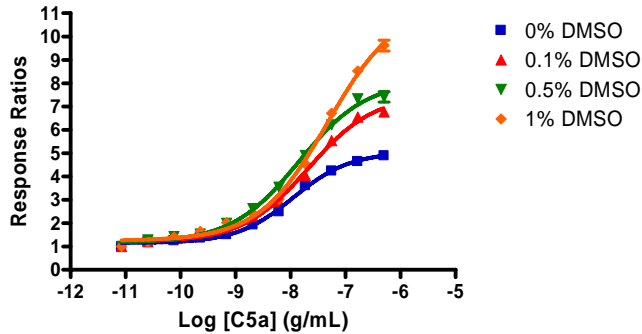
**Figure 6— C5a dose response with 1, 1.5, and 2 hour substrate loading times.**



GeneBLAzer® C5AR1-Gα15-NFAT-*bla* CHO-K1 cells (10,000 cells/well) were plated the day before the assay in a 384-well black-walled tissue culture assay plate. C5a (R & D Systems 2037-C5) was then added to the plate over the indicated concentration range in 0.5% DMSO for 5 hours and then loaded for 1, 1.5 or 2 hours with LiveBLAzer™-FRET B/G Substrate. Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and the Response Ratios for each substrate loading time plotted against the indicated concentrations of C5a (n=8 for each data point).

### Assay Performance with Variable DMSO Concentration

Figure 7 – C5a dose response with 0, 0.1, 0.5 and 1% DMSO



GeneBLAzer® C5AR1-Gα15-NFAT-*bla* CHO-K1 cells (10,000 cells/well) were plated the day before the assay in a 384-well assay plate. C5a (R & D Systems 2037-C5) was then added to the plate over the indicated concentration range. DMSO was added to separate wells at concentrations from 0% to 1%. Cells were stimulated for 5 hrs with agonist and loaded for 2 hours with LiveBLAzer™-FRET B/G Substrate. Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and the Response Ratios are shown for each DMSO concentration against the indicated concentrations of C5a (n=8 for each data point).

## References

1. Monk, P.N.; Scola, A.M.; Madala, P.; and Fairlie, D.P. (2007). **Function, structure and therapeutic potential of complement C5a receptors.** British Journal of Pharmacology: advanced online publication July 2, 2007.
2. Friedberger E (1910). **Weitere untersuchungen uber Eisissanaphylaxie: IV.** Mitteilung. Immunitaetaforsch Exp Ther 4: 636–690.
3. Snyderman R, Phillips J, Mergenhausen SE (1970). **Polymorphonuclear Leukocyte Chemotactic Activity in Rabbit Serum and Guinea Pig Serum Treated with Immune Complexes: Evidence for C5a as the Major Chemotactic Factor.** Infect Immun 1: 521–525.
4. Becker EL (1972). **The relationship of the chemotactic behavior of the complement-derived factors, C3a, C5a, and C567, and a bacterial chemotactic factor to their ability to activate the proesterase 1 of rabbit polymorphonuclear leukocytes.** J Exp Med 135: 376–387.
5. Snyderman R, Pike MC, Mccarley D, Lang L (1975). **Quantification of mouse macrophage chemotaxis *in vitro*: role of C5 for the production of chemotactic activity.** Infect Immun 11: 488–492.
6. Boulay F, Mery L, Tardif M, Brouchon L, Vignais P (1991). **Expression cloning of a receptor for C5a anaphylatoxin on differentiated HL-60 cells.** Biochemistry 30: 2993–2999.
7. Gerard NP, Gerard C (1991). **The chemotactic receptor for human C5a anaphylatoxin.** Nature 349: 614–617.
8. Ohno M, Hirata T, Enomoto M, Araki T, Ishimaru H, Takahashi TA (2000). **A putative chemoattractant receptor, C5L2, is expressed in granulocyte and immature dendritic cells, but not in mature dendritic cells.** Mol Immunol 37: 407–412.