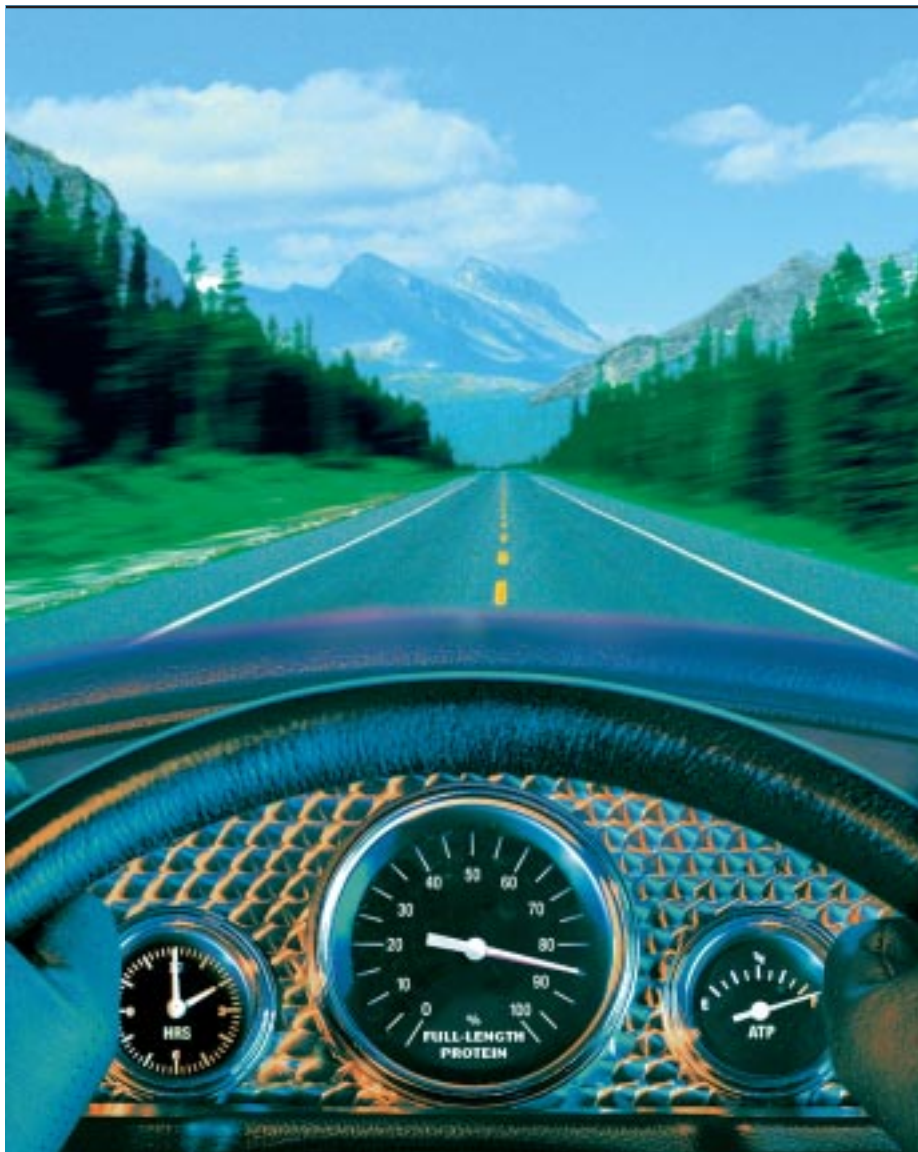




## Accelerate **Production of Active Protein** with Expressway™ Plus

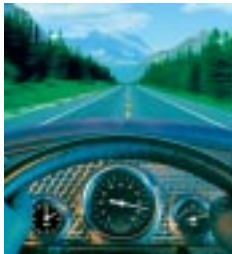


With the Expressway™ Plus System you can:

- Produce full-length, functional protein in just 2 hours
- Eliminate the hassle and cost of cell maintenance
- Scale the reaction to achieve microgram to milligram levels of protein

**NEW**

# High-performance protein expression



Traditional protein expression systems involve expressing recombinant proteins in intact eukaryotic or prokaryotic cells. Although effective, these methods can be both time-consuming and technically challenging. The Expressway™ Plus Expression System eliminates these problems by allowing you to directly synthesize high yields of recombinant protein in a single reaction tube in just 2 hours. With Expressway™ Plus, you'll save hours of time and labor and produce the protein yields you need for downstream functional analysis.

## Simple, speedy cell-free system

Conventional protein production using intact cells is time-consuming, requiring the transformation of a plasmid containing the gene of interest, growth and maintenance of cells, and optimization of expression parameters. The Expressway™ Plus System eliminates these steps by allowing you to *in vitro* transcribe and translate target DNA to protein in three easy steps (Figure 1). Unlike eukaryotic systems in which transcription and translation occur sequentially, the

Expressway™ Plus System uses an *E. coli* extract that allows simultaneous transcription and translation. There's no need to perform separate optimization for these reactions. Because the Expressway™ Plus System is a cell-free system, background protein synthesis from endogenous RNA is minimal. You'll get fast, efficient protein expression from your gene of interest without the hassle, time, and cost associated with cell-based systems (Figure 2).

Figure 1 – Active protein in three easy steps

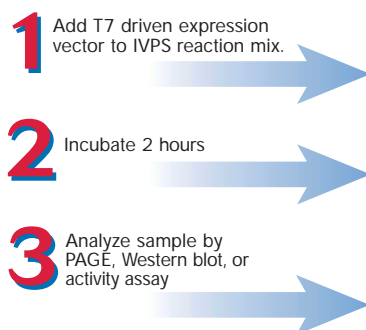


Figure 2 – Fast *in vitro* expression saves time

System	Time
Bacterial	24–36 hours
Yeast	36–48 hours
Insect	48–60 hours
Mammalian	48–60 hours
<b>Expressway™ Plus</b>	<b>2 hours</b>

## System flexibility

The Expressway™ Plus Expression System is an open system that allows you to:

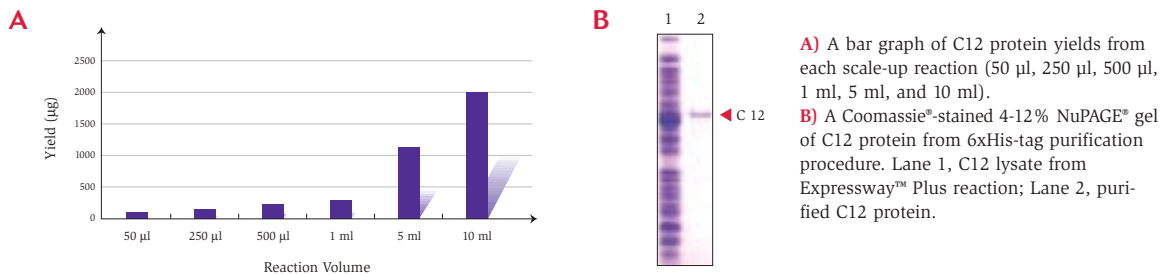
- Use your own T7 promoter-driven expression vector, or use a specially designed Expressway™ Plus vector
- Incorporate additional protein-specific co-factors, chaperones, and detergents to the reaction mix, as desired
- Express genes toxic to *in vivo* systems
- Easily perform high-throughput synthesis of proteins
- Perform co-translational protein labeling on the N- or C-terminus
- Rapidly express proteins for Protein Truncation Testing
- Perform rapid and simple mutational analysis

## Scale-up for more

Traditional cell-free expression systems produce only low levels of recombinant protein. The Expressway™ Plus System enables you to scale your expression—from micrograms to milligrams—to suit your down-

stream needs (Figure 3). Whichever you choose, you'll have the protein yield you need in a simple two-hour reaction.

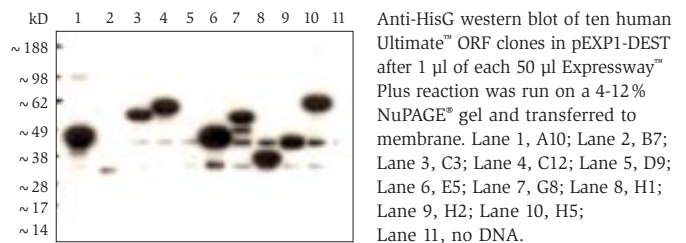
**Figure 3 – Increase in reaction volume: C12 expression and purification in the Expressway™ Plus System**



## Express a range of proteins

Using the Expressway™ Plus System, you can quickly and simply express proteins from a variety of origins. Easily go from clone to protein in straightforward and reliable steps. To demonstrate, a 96-well plate of Ultimate™ ORF human clones (kinases) was recombined into the pEXP1-DEST vector to generate expression clones. Each expression clone was then used as the template in a standard 50 µl Expressway™ Plus reaction. Expression results indicate that greater than 90% of the human kinases were expressed (Figure 4), proving that the Expressway™ Plus System is an ideal method for fast and convenient protein expression.

**Figure 4 – Ten Human Ultimate™ ORF clones expressed in the Expressway™ Plus System**

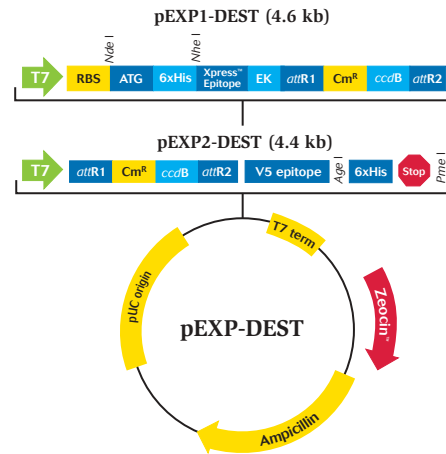


## Optimized vector options

You can save time and effort with the Expressway™ Plus Gateway® vectors\*. These vectors contain the necessary sequence configuration and optimal spacing for expression (Figure 5). Simply recombine your gene of interest (GOI) from any Gateway® entry vector into the Expressway™ Plus destination vector in a fast, one-hour reaction. If your GOI and entry vector do not contain a ribosome-binding site, select the pEXP1-DEST vector. If a ribosome-binding site is present, choose the pEXP2-DEST vector.

\*For more information on Gateway® Technology see the next page and [www.invitrogen.com/gateway](http://www.invitrogen.com/gateway)

Figure 5 – pEXP1-DEST and pEXP2-DEST vectors



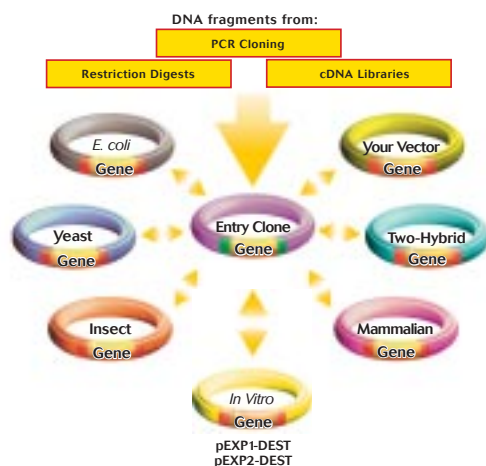
Use of the Gateway® destination vector included in the Expressway™ Plus kit requires transferring the clone of interest from a compatible Gateway® entry vector.

## Gateway® route to expression

Gateway® Technology offers the most comprehensive route to functional gene analysis and protein expression. This versatile system gives you nearly limitless access to other expression options, and is the best choice when your experimental design includes analysis of your protein in more than one system (Figure 6). A

choice of optimized, supercoiled Gateway® destination vectors is available in the Expressway™ Plus kits. Whether you use the combined power of Expressway™ and Gateway®, or use another T7 vector, Expressway™ Plus puts you in the driver's seat for protein expression.

Figure 6 – Gateway® Technology overview



1. Create a Gateway® entry clone by PCR or traditional cloning.
2. Mix an entry clone with the pEXP-DEST vector of your choice.
3. Use in the Expressway™ Plus System.

Once a gene is cloned into an entry vector, you can then move the DNA fragment into one or more destination vectors simultaneously.

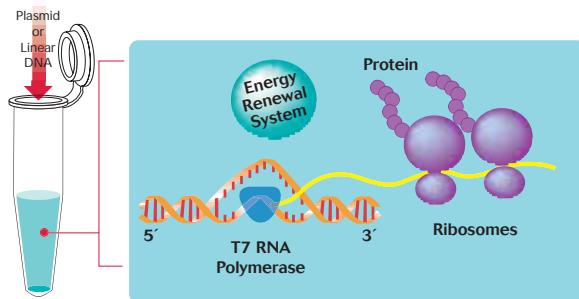
## Efficient single-tube format

The Expressway™ Plus System provides all the components you require for optimal cell-free protein production, including:

- An optimized *E. coli* extract containing the cellular machinery required to drive transcription and translation
- IVPS Plus *E. coli* reaction buffer containing the amino acids (except methionine) necessary for translation, as well as an ATP regenerating system for energy

Simply mix reaction buffer, methionine, T7 Enzyme Mix, and your DNA template (driven by a T7 promoter) with the *E. coli* extract in one tube. As the DNA template is transcribed, the 5' end of the mRNA is bound by ribosomes and undergoes translation as the 3' end of the template is still being transcribed (Figure 7). With the Expressway™ Plus System, you'll have high yields of functional protein in just two hours.

Figure 7 – How the Expressway™ Plus System works



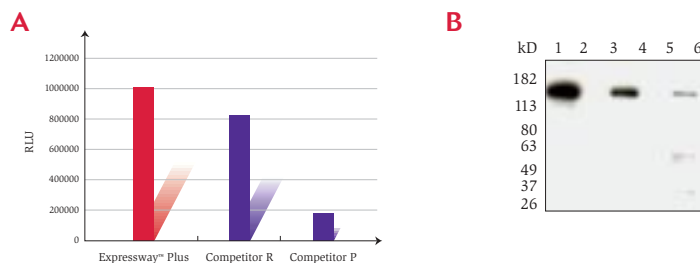
As a DNA template driven by a T7 promoter is transcribed, the 5' end of the mRNA is bound by ribosomes and undergoes translation. A specially engineered ATP energy renewal system coupled with early ribosome binding for transcript stability results in high protein yields.

## Functional protein made easy

The optimized Expressway™ Plus System yields enough full-length, active protein for downstream functional analysis in a single experiment. When compared to competitors' cell-free expression systems, the Expressway™

Plus System provides more full-length protein and more active protein (Figure 8). Using the Expressway™ Plus System, you'll quickly and easily produce the protein you need for downstream analysis.

Figure 8 – Comparison of Expressway™ Plus with other available *in vitro* systems



**A)** Endpoint  $\beta$ -galactosidase activity assay of 1  $\mu$ l of 2 hour-50  $\mu$ l *in vitro* synthesis of competitor R, P, and Expressway™ Plus reactions. **B)** Autoradiograph of 1.25  $\mu$ l of each standard 50  $\mu$ l reaction with or without pEXP1-GW-LacZ control DNA loaded onto a 4-12% Tris-Glycine SDS PAGE gel. Lane 1: Expressway™ Plus reaction plus DNA; Lane 2: Expressway™ Plus reaction no DNA; Lane 3: R reaction plus DNA; Lane 4: R reaction no DNA; Lane 5: P reaction plus DNA; Lane 6: P reaction no DNA.

## Versatile and efficient

With the Expressway™ Plus System, the road to discovery is full of possibilities. Cell-free, *in vitro* expression with Expressway™ Plus puts functional genomics and proteomics research in the fast lane. For diverse applications in basic research, drug discovery, molecular diagnostics, and high-throughput screening,

Expressway™ Plus offers the yield and speed today's researchers need. Whether you're performing functional characterization of proteins, verification of cloned gene products, molecular interaction detection or production of toxic proteins, Expressway™ Plus leads the way.

## Optimized performance

The Expressway™ Plus System is optimized for high-yield *in vitro* protein synthesis of functional protein.

The kit includes the components you need to get started (Table 1). Call Invitrogen and order today.

**Table 1 – Expressway™ Plus Kit components**

IVPS Plus <i>E. coli</i> Extract
2.5X IVPS Plus Reaction Buffer
RNase A
T7 Enzyme Mix
Methionine
Reaction tubes
Expression control plasmids
Expression vector when indicated: pEXP1-DEST or pEXP2-DEST

Product	Quantity	Cat. no.
Expressway™ Plus Expression System		
without vector	20 rxns	K9900-10
with pEXP1-DEST	20 rxns	K9900-20
with pEXP2-DEST	20 rxns	K9900-30
pEXP1-DEST Gateway® Vector Kit	6 µg	V960-01
pEXP2-DEST Gateway® Vector Kit	6 µg	V960-02

### Reference

1. Studier, F.W., Rosenberg, A.H., Dunn, J.J., and Dubendorff, J.W. (1990) *Meth. Enzymol.* 185, 60-89.



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