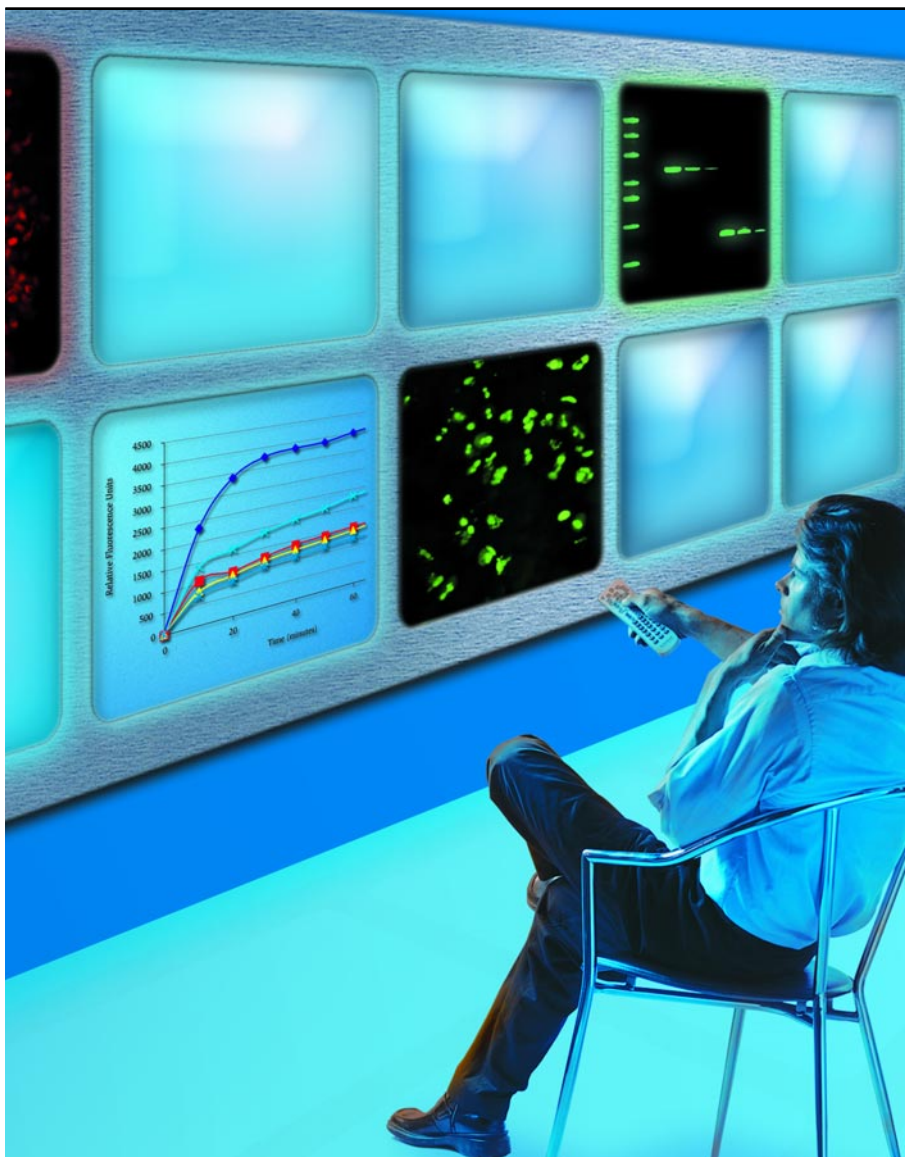


## One tool, multiple perspectives

Use Lumio™ Technology for rapid, reliable, and versatile protein detection



### Lumio™ Protein Detection Technology provides:

- Sensitive localization of proteins in living cells
- Real-time monitoring of protein expression
- Fastest, easiest in-gel detection of recombinant proteins

# The fastest and easiest protein detection technology

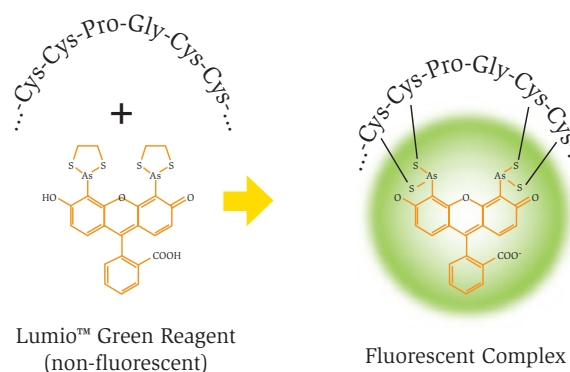


Detection of protein expression and cellular location are common bottlenecks in today's laboratory. The versatile Lumio™ Technology platform is designed to eliminate these bottlenecks. Using a small, novel recognition sequence and target-specific, fluorescent labeling reagents, you'll precisely monitor protein expression in a variety of host systems.

## Overview of Lumio™ Technology

The Lumio™ recognition sequence is a small, six-amino acid sequence (Cys-Cys-Pro-Gly-Cys-Cys). This unique sequence rarely appears in endogenous proteins—providing precise detection of proteins with this fusion tag. The Lumio™ detection reagents bind this sequence with high specificity and affinity, resulting in a bright fluorescent signal (Figure 1). A number of Lumio™ vectors and reagents are available, allowing you to take advantage of this unique technology for a variety of applications in multiple host systems.

Figure 1 - The Lumio™ Reagents become fluorescent upon binding to the Lumio™ tag



## A wide range of applications

Lumio™ Technology is the fastest, easiest way to detect recombinant proteins across multiple applications. With Lumio™ Technology, you'll get:

- **In-cell protein localization**—less background and higher sensitivity than other detection methods
- **Real-time protein production analysis**—easily evaluate the rate of protein synthesis
- **Immediate in-gel protein detection**—eliminate protein staining and time-consuming western blot procedures

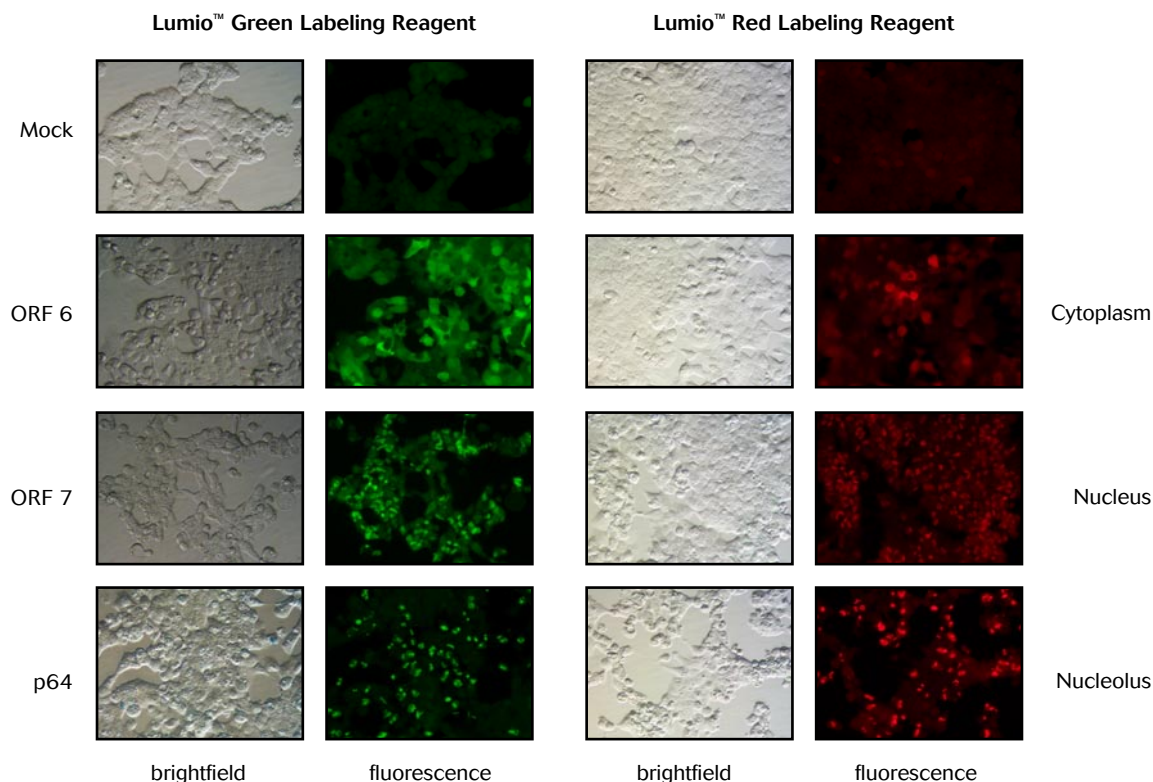
## Sensitive localization of proteins in mammalian cells

Lumio™ Technology provides an effective way to localize your protein of interest in live mammalian cells (Figure 2). By simply expressing your protein of interest fused with the Lumio™ tag and labeling live cells with Lumio™ In-Cell Labeling Reagents, you'll be able to rapidly track the location of your protein. You can easily perform single color labeling experiments with the Lumio™ Green In-Cell Labeling Kit.

Choose both the Lumio™ Green and Lumio™ Red In-Cell Labeling Kits if you wish to perform dual labeling assays (e.g. pulse-chase experiments) to study such processes as protein assembly, protein internalization, and turnover. You'll get:

- Proper protein activity—the small tag is unlikely to disrupt protein function
- Time savings—no need for laborious fixing and antibody labeling protocols
- Assay conditions that represent real-time physiological cellular conditions to eliminate possible fixing artifacts

Figure 2 - Sensitive protein localization with the Lumio™ In-Cell Labeling Kits



*In vivo* labeling of Lumio™ ORFs. GripTite™ 293 MSR cells were transiently transfected with no DNA (mock), pcDNA™6.2/nLumio™-GW/ORF 6 (ORF 6), pcDNA™6.2/nLumio™-GW/ORF 7 (ORF 7), or pcDNA™6.2/nLumio™-GW/p64 (p64). Forty-eight hours post-transfection, cells were labeled with either 2.5 μM Lumio™ Green In-Cell Labeling Reagent (left panels) or 2.5 μM Lumio™ Red In-Cell Labeling Reagent (right panels) and visualized under a fluorescence microscope. Lumio™ Green was detected with a standard FITC filter (excitation wavelength 460-490 nm) and Lumio™ Red was detected with a standard Texas Red® filter (excitation wavelength 545-550 nm).

## Real-time expression detection

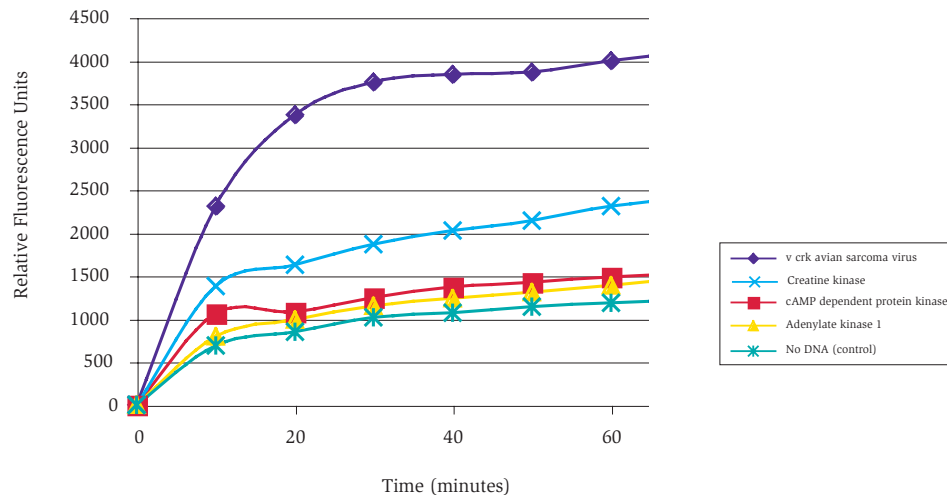
Combining Lumio™ Technology with Expressway™ Plus cell-free expression, you can monitor protein expression in real time.

This enables you to:

- Confirm protein expression without gels
- Evaluate transcription/translation regulators
- Screen for expression in a high-throughput format

For real-time analysis, simply add the Lumio™ Green Detection Reagent directly to the reaction mix in the Expressway™ Plus Expression System. You can detect protein synthesis on a fluorometer in real time—allowing you to evaluate reaction kinetics without interrupting the mixture by taking aliquots (Figure 3).

**Figure 3 - Real-time detection of protein production with Lumio™ Technology**



Using the Lumio™ Green Detection Reagent, real-time expression of Lumio™ fusion proteins was measured directly from 50 µl *in vitro* protein synthesis reactions in a 96-well plate at 37°C using a 96-well plate reader. The excitation wavelength was set at 500 nm, while emission was monitored at 535 nm. Readings were taken at 10 minute intervals over a one hour incubation period.

## Immediate in-gel detection of recombinant protein

Confirm recombinant protein expression quickly and conveniently with Lumio™ Technology. No need to perform tedious gel staining or western blotting procedures. The Lumio™ Green Detection Reagent labels Lumio™ fusion proteins, allowing you to immediately visualize proteins directly in a polyacrylamide gel.

Simply add the Lumio™ Green Detection Kit reagents to your protein sample prior to electrophoresis, and run the gel. Then visualize with a UV transilluminator, equipped with a standard camera, or a laser-based scanner (Figure 4).

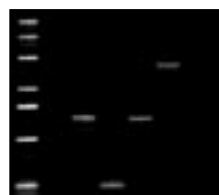
**Figure 4 - Immediate in-gel detection of recombinant proteins with Lumio™ Technology**



1. Add the Lumio™ Green Detection Reagent and the optimized Lumio™ Sample Buffer to your protein sample, then add Lumio™ In-Gel Detection Enhancer to your sample mix.



2. Load and electrophorese your protein samples.



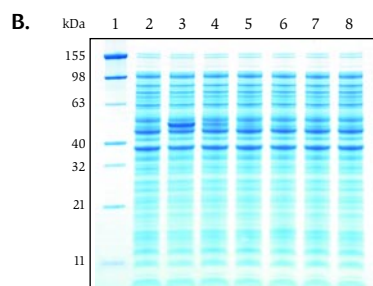
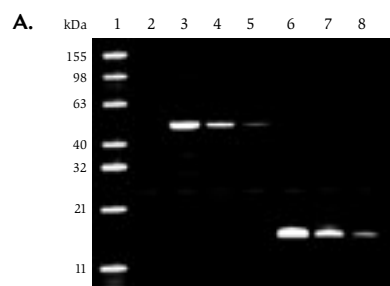
3. Immediately after electrophoresis, visualize your Lumio™ fusion protein bands with a UV transilluminator, equipped with a standard camera, or a laser-based scanner.

## Highly specific and sensitive

With the Lumio™ Green Detection Kit, you can detect nanogram levels of Lumio™ fusion protein (Figure 5). The high sensitivity and specificity of the Lumio™ Green Detection Reagent enables you to identify Lumio™ fusion proteins that would normally be impossible to distinguish

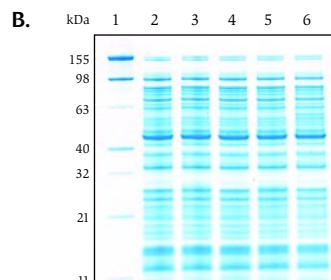
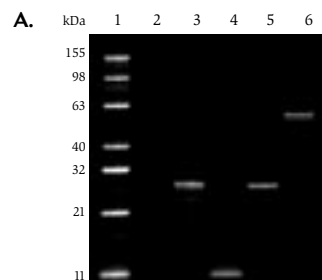
from the endogenous protein population by Coomassie® staining (Figure 6). After documenting the Lumio™ Green fluorescence signal, total protein staining can be performed.

**Figure 5 - Sensitive detection of Lumio™ fusion protein using the Lumio™ Green Detection Kit**



Protein samples were labeled using the Lumio™ Green Detection Kit and electrophoresed on a NuPAGE® Novex 4-12% Bis-Tris gel. The Lumio™ Green signal (A). Total protein detection with SimplyBlue™ SafeStain (B). Arrows indicate Lumio™ fusion proteins.  
Lane 1: BenchMark™ Fluorescent Protein Standard  
Lane 2: BL21 Star™ *E. coli* extract (negative control)  
Lane 3-5: 1200 ng, 240 ng, 48 ng, respectively, of pure 48 kDa Lumio™ fusion protein mixed into BL21 Star™ *E. coli* extract  
Lane 6-8: 225 ng, 45 ng, 9 ng, respectively, of pure 15 kDa Lumio™ fusion protein mixed into BL21 Star™ *E. coli* extract

**Figure 6 - Specific detection of Lumio™ fusion proteins using the Lumio™ Green Detection Kit**



Several Lumio™ fusion proteins were expressed using the Expressway™ Plus Expression System. Protein samples were labeled using the Lumio™ Green Detection Kit and electrophoresed on a NuPAGE® Novex 4-12% Bis-Tris gel. The Lumio™ Green signal (A). Total protein detection with SimplyBlue™ SafeStain (B). Arrows indicate Lumio™ fusion proteins.  
Lane 1: BenchMark™ Fluorescent Protein Standard  
Lane 2: Negative expression control  
Lanes 3-6: Lumio™ fusion proteins human Krev, human c-Jun leucine zipper domain, CAT, and mouse E2F1, respectively



## Choose your host system

A variety of vectors containing the Lumio™ tag are currently available, allowing you to take advantage of Lumio™ Technology in a cell-free, *E. coli*, or mammalian system. Simply place your gene of interest into a vector and express it as a fusion to the Lumio™ tag. Designed to facilitate analysis for different applications, vectors carrying the Lumio™ tag use Gateway® Technology for easy transfer of your gene without subcloning.

You can select one application to begin your analysis and then shuttle your gene to other vectors designed for different applications (Table 1). Visit [www.invitrogen.com/gateway](http://www.invitrogen.com/gateway) for more details. Champion™ pET expression vectors are also available as Directional TOPO® vectors. You can quickly and easily clone your blunt-end PCR product in a 5-minute reaction.

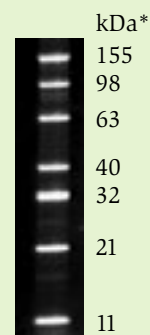
**Table 1 - Lumio™ Technology applications**

Protein detection application	Expression System	Lumio™ System	Host benefits	Lumio™ Kit
<b>Protein detection and localization in live mammalian cells (see page 3)</b>	Mammalian	Gateway® vectors with Lumio™ Technology (See page 7)	Expression of eukaryotic proteins	Lumio™ Green or Lumio™ Red In-Cell Labeling Kits
<b>Real-time monitoring of protein expression (see page 4)</b>	Cell free	Expressway™ Plus Expression System with Lumio™ Technology (See page 8)	Rapid, simple protein production	Lumio™ Green Detection Kit
<b>In-gel detection (see page 5)</b>	Cell free	Expressway™ Plus Expression System with Lumio™ Technology	Rapid, simple protein production	Lumio™ Green Detection Kit
	<i>E. coli</i>	Champion™ pET Expression System with Lumio™ Technology (See page 9)	Highest-level protein production	Lumio™ Green Detection Kit
	Mammalian	Gateway® vectors with Lumio™ Technology	Expression of eukaryotic proteins	Lumio™ Green Detection Kit

### Direct marker visualization with a UV transilluminator

BenchMark™ Fluorescent Protein Molecular Weight Standard allows direct marker visualization after SDS-PAGE with a UV light box or commonly used laser-based scanner systems. Each of the standard proteins is conjugated with Alexa Fluor® 488, a superior fluorescent dye. Seven sharp bands resolve clearly on NuPAGE® Gels or Novex® Tris-Glycine Gels, suitable for easy molecular weight estimation of fluorescently-labeled proteins. Using the ready-to-load BenchMark™ Fluorescent Standard, you'll be able to conveniently size your recombinant Lumio™ proteins on SDS-PAGE Gels.

\* Apparent MW values vary depending on gel system



5 µl of the BenchMark™ Fluorescent Standard was resolved on a NuPAGE® 4-12% Bis-Tris Gel/MES then visualized with a UV transilluminator.

## Reliable vectors for high-level mammalian expression

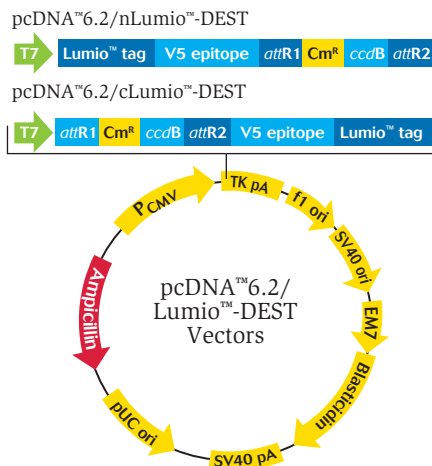
pcDNA™ vectors are the most cited and reliable vectors known for high-level mammalian expression.

Lumio™ Technology makes them even more effective. The Mammalian Lumio™ Gateway® vectors (Figure 1) offer the following features:

- Lumio™ tag for accurate *in vitro* or *in vivo* protein detection
- CMV promoter for high-level constitutive expression
- *attR* sites for efficient recombination with Gateway® entry clones
- Blasticidin resistance gene for fast, efficient selection

Use Mammalian Lumio™ Gateway® vectors for reliable and consistent protein expression detection and localization in mammalian cells. You have the opportunity to test both N- and C-terminal Lumio™ vectors to determine which fusion is optimal for your protein of interest. In addition, you may choose the Lumio™ Green or Lumio™ Red In-Cell Labeling Kits or try the Lumio™ Dual Labeling Kit for your pulse-chase studies.

Figure 1 - Lumio™ pcDNA™ vectors



### Product

Product	Quantity	Cat. no.
Mammalian Lumio™ Gateway® Vectors		
with Lumio™ Green In-Cell Labeling Kit	1 kit	12589-016
with Lumio™ Red In-Cell Labeling Kit	1 kit	12589-024
with Lumio™ Dual Green and Red In-Cell Labeling Kits	1 kit	12589-032
Lumio™ Red In-Cell Labeling Kit	1 kit	12589-040
Lumio™ Green In-Cell Labeling Kit	1 kit	12589-057

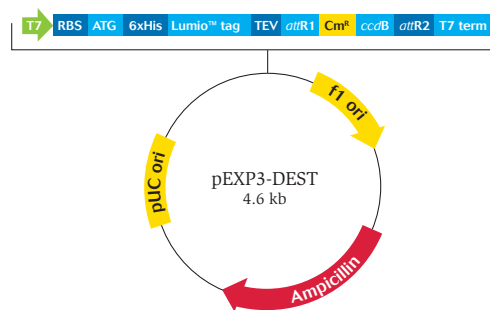
Each Mammalian Lumio™ Gateway® kit includes pcDNA™6.2/nLumio™-DEST, pcDNA™6.2/cLumio™-DEST, and pcDNA™6.2/nLumio™-GW/p64 control. Each Lumio™ In-Cell Labeling Kit contains 40 µl of a 2mM stock of Lumio™ Labeling Reagent.

## High yields of active protein in 2 hours in a cell-free system

The Expressway™ Plus Expression System\* is a cell-free, coupled transcription/translation system that generates active protein in two hours (Figure 1). The Lumio™ Expressway™ vector (Figure 2) features:

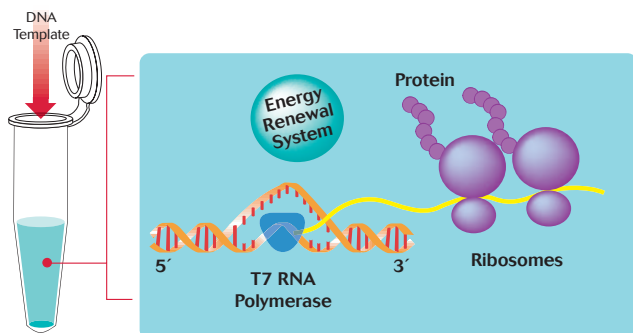
- Lumio™ tag for real-time and in-gel protein detection
- 6xHis tag for rapid protein purification with ProBond™ Nickel-Chelating Resin
- TEV cleavage site for efficient removal of the fusion tag following purification
- T7 promoter, ribosome binding site, and T7 terminator optimally spaced for cell-free protein expression

Figure 2 - Lumio™ Expressway™ vector



Use the Expressway™ Plus Expression System with Lumio™ Technology for real-time transcription/translation analysis, protein characterization, verification and functional analysis, production of toxic or labeled proteins, and high-throughput screening.

Figure 1 - Expressway™ Plus Expression System



The Expressway™ Plus Expression System is a cell-free, coupled transcription/translation system that generates full-length, active protein in two hours. 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.

### Product

Expressway™ Plus Expression System with Lumio™ Technology  
 with pEXP3-DEST  
 without vector  
 pEXP3-DEST

### Quantity

20 rxns  
 20 rxns  
 6 µg

### Cat. no.

K9900-70  
 K9900-60  
 V960-03

The Expressway™ Plus Expression System with Lumio™ Technology includes IVPS Lumio™ *E. coli* Extract, IVPS Plus *E. coli* Reaction Buffer, RNase A, T7 Enzyme Mix, Methionine, water, reaction tubes, 6 µg of the pEXP3-DEST vector, control plasmid and Lumio™ Green Detection Kit for 20 real-time reactions or 100 in-gel applications, and Benchmark™ Fluorescent Protein Standard provides sufficient reagent for 25 mini-gel applications (included only in the system with pEXP3-DEST).

\* To learn more about the Expressway™ Plus Expression System, visit [www.invitrogen.com/expressway](http://www.invitrogen.com/expressway).



## Achieve the highest protein yields in *E. coli*

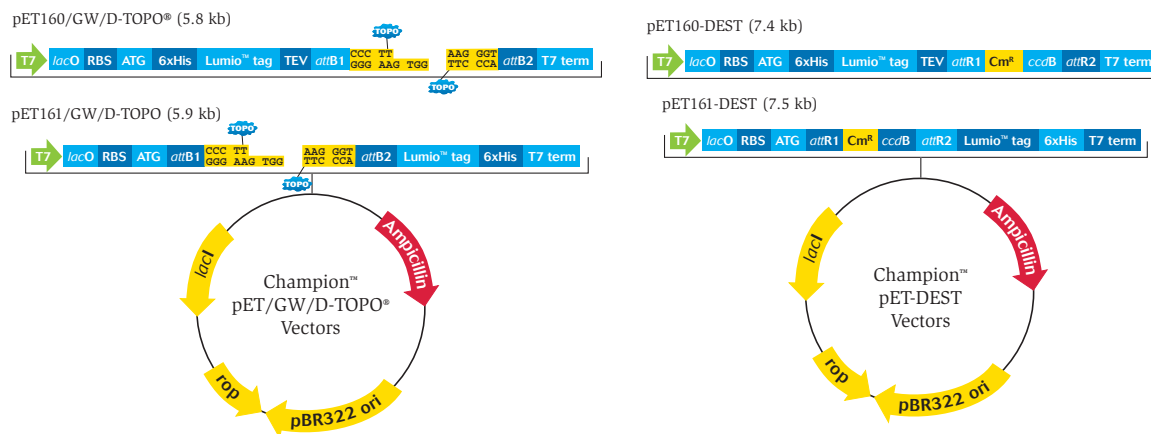
The Champion™ pET Expression System produces the highest possible protein yields in *E. coli*. By combining Champion™ pET Expression Vectors with BL21 Star™ *E. coli*, this system generates protein yields two- to ten-fold greater than any other expression system. You can directionally clone your gene of interest into the Champion™ pET vectors using fast, efficient TOPO® Cloning Technology or a simple, Gateway® recombination reaction\*. All Lumio™ Champion™ pET vectors (Figure 1) feature:

- T7lac promoter for inducible, high-level expression in bacteria

- Lumio™ tag for in-gel protein detection (expressed as an N- or C-terminal fusion)
- 6xHis tag for rapid protein purification with ProBond™ Nickel-Chelating Resin
- TEV cleavage site for efficient removal of the Lumio™ tag following purification (N-terminal fusion vectors only)

Use the Champion™ pET Expression System to produce protein for functional protein analysis, antibody production, X-ray crystallography, and bioproduction.

Figure 1 - Champion™ pET vectors with Lumio™ Technology



### Product

Product	Quantity	Cat. no.
Champion™ pET160 Directional TOPO® Expression Kit with Lumio™ Technology	20 rxns	K160-01
Champion™ pET161 Directional TOPO® Expression Kit with Lumio™ Technology	20 rxns	K161-01
Champion™ pET160 Gateway® Expression Kit with Lumio™ Technology	20 rxns	12583-035
Champion™ pET161 Gateway® Expression Kit with Lumio™ Technology	20 rxns	12583-043

Each Champion™ pET Gateway® Expression Kit is provided as a complete expression system. The Gateway® Expression box contains 6 µg of the Gateway® destination vector and 100 ng of the Gateway® expression control. The One Shot™ TOP10 box contains twenty-one 50 µl aliquots of chemically competent *E. coli*, S.O.C. medium, and a control plasmid. The One Shot™ BL21 Star™ (DE3) box contains twenty-one 50 µl aliquots of chemically competent *E. coli*, S.O.C. medium, and a control plasmid. The Lumio™ Green Detection Kit provides sufficient detection reagent for 100 in gel applications. The Benchmark™ Fluorescent Protein Standard provides sufficient reagent for 25 mini-gel applications.

Each Champion™ pET Directional TOPO® Expression Kit is provided as a complete expression system. The Directional TOPO® Expression box contains topoisomerase I-activated Champion™ pET vector; sterile water; dNTPs; 10X PCR Buffer; salt solution; control template and primers; primers for sequencing or PCR screening; and an expression control. The One Shot™ TOP 10 box contains twenty-one 50 µl aliquots of chemically competent *E. coli*, S.O.C. medium, and a control plasmid. The One Shot™ BL21 Star™ (DE3) box contains twenty-one 50 µl aliquots of chemically competent *E. coli*, S.O.C. medium, and a control plasmid. The Lumio™ Green Detection Kit provides sufficient detection reagent for 100 in-gel applications. The Benchmark™ Fluorescent Protein Standard provides sufficient reagent for 25 mini-gel applications.

\* For more information on TOPO® Cloning or Gateway® Technology, visit [www.invitrogen.com](http://www.invitrogen.com).

## Easy detection, powerful expression

Use Lumio™ Technology for fast, easy, and accurate protein detection. Lumio™ Kits are available for cell-free, *E. coli*, and mammalian expression systems. Call and order today.

### Summary of Lumio™ Technology products

#### Mammalian Lumio™ Gateway® Vectors

Product	Quantity	Cat. no.
Mammalian Lumio™ Gateway® Vectors		
with Lumio™ Green In-Cell Labeling Kit	1 kit	12589-016
with Lumio™ Red In-Cell Labeling Kit	1 kit	12589-024
with Lumio™ Dual Green and Red In-Cell Labeling Kits	1 kit	12589-032

#### Expressway™ Plus Expression System with Lumio™ Technology

Product	Quantity	Cat. no.
Expressway™ Plus Expression System With Lumio™ Technology		
with pEXP3-DEST <sup>+</sup>	20 rxns	K9900-70
without vector <sup>++</sup>	20 rxns	K9900-60
pEXP3-DEST	6 µg	V960-03

#### Champion™ pET Gateway® Expression Kits with Lumio™ Technology

Product	Quantity	Cat. no.
Champion™ pET160 Gateway® Expression Kit <sup>+</sup>	20 rxns	12583-035
Champion™ pET161 Gateway® Expression Kit <sup>+</sup>	20 rxns	12583-043

#### Champion™ pET Directional TOPO® Expression Kits with Lumio™ Technology

Product	Quantity	Cat. no.
Champion™ pET160 Directional TOPO® Expression Kit <sup>+</sup>	20 rxns	K160-01
Champion™ pET161 Directional TOPO® Expression Kit <sup>+</sup>	20 rxns	K161-01

#### Lumio™ Detection Reagents and In-Gel Protein Standard

Product	Application	Cat. no.
Lumio™ Green Detection Kit*	Specific in-gel or <i>in vitro</i> detection of recombinant proteins	LC6090
BenchMark™ Fluorescent Protein Standard	Direct marker visualization under UV and molecular weight sizing for proteins labeled with the Lumio™ Green Detection Reagent	LC5928
Lumio™ Green In-Cell Labeling Kit	Detection of recombinant proteins in living mammalian cells	12589-057
Lumio™ Red In-Cell Labeling Kit	Detection of recombinant proteins in living mammalian cells	12589-040

\*LC6090 includes one vial of Lumio™ Green Detection Reagent, five vials of Lumio™ Gel Sample Buffer, and one vial of Lumio™ In-Gel Detection Enhancer. Each kit provides sufficient reagents for 100 in-gel detection applications or 20 *in vitro* transcription-translation labeling reactions.

\*Kits include the Lumio™ Green Detection Kit and BenchMark™ Fluorescent Protein Standard

\*\*Kit includes the Lumio™ Green Detection Kit

**NOTES:**

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