Generate pET constructs in less time

Express native or tagged proteins from one vector

PanVera acquisition increases your resources

Improve your expression profiling

Free your hands

BaculoDirect™ Baculovirus Expression System requires less hands-on time
Free your hands with simplified baculovirus expression

The BaculoDirect™ Baculovirus Expression System makes baculovirus expression faster and easier than ever before—saving you hours of time when compared to other baculovirus systems. With the BaculoDirect™ System, you’ll get rapid results with less effort and fewer steps.

**unique in speed**
Traditional baculovirus systems require bacterial transformation and isolation of a large bacmid or co-transfection of baculovirus linear DNA and a transfer vector into insect cells. The BaculoDirect™ System gives you rapid results by eliminating these time-consuming steps. From initial transfection to isolation of your baculovirus stock takes only 8 hours of hands-on time—less than half the time required with traditional baculovirus systems.

**simplified cloning**
The BaculoDirect™ System utilizes Gateway® Technology to streamline your research. The specially engineered BaculoDirect™ Linear DNA (baculovirus genome) contains attR sites for efficient recombination with a Gateway® entry clone containing your gene of interest. Just mix the entry clone containing your gene of interest with the BaculoDirect™ Linear DNA and LR Clonase® Enzyme Mix for one hour at room temperature. The resulting reaction mix contains the recombinant baculovirus carrying your gene of interest. Use this reaction mix to directly transfect insect cells. After seventy-two hours of growth, you’ll have your recombinant baculovirus ready for expression or amplification. Baculovirus expression has never been easier.

**analyze more**
Unlike other baculovirus systems, the BaculoDirect™ System is truly amenable to high-throughput expression screening. Gateway® entry clones and the BaculoDirect™ Linear DNA can be arrayed in multiple wells, enabling you to perform the LR reaction, transfection, and infection of insect cells in multi-well plates. This allows you to analyze multiple proteins simultaneously. You’ll save time and get your results faster.

**parent-free virus**
The BaculoDirect™ Linear DNA includes two built-in time-saving checks—the thymidine kinase (TK) gene and lacZ gene—to ensure the isolation of a pure baculovirus stock (figure 1). During the LR reaction with your Gateway® entry clone, the TK and lacZ genes are recombined out as by-products of the reaction. Cells are then placed under the selection of ganciclovir, a drug that selects against virus containing the TK gene. Ganciclovir efficiently eliminates any remaining parental, non-recombinant virus. The purity of the viral stock can be quickly determined by staining cells with β-galactosidase to reveal any remaining lacZ-containing parental, non-recombinant virus. With the BaculoDirect™ System, you’ll have a pure virus stock in only one week and eliminate the need for multiple rounds of plaque purification to isolate recombinant virus.

**hands-free expression**
Save time and get to your expression results faster with the BaculoDirect™ Baculovirus Expression System. Call and order today.

---

**figure 1 - engineered BaculoDirect™ Linear DNA for simplified, fast baculovirus expression**

**Product**  | **Quantity** | **Cat. no.**
--- | --- | ---
BaculoDirect™ Expression Kit† | 5 reactions | 12562-013
BaculoDirect™ Transfection Kit†† | 5 reactions | 12562-039

† To create a Gateway® entry clone, clone your gene of interest into a Gateway® entry vector using PCR cloning or traditional restriction enzyme digest and ligation. For more information, visit www.invitrogen.com/gateway.
† The BaculoDirect™ Expression Kit includes BaculoDirect™ Linear DNA, pENTR™/CAT (Gateway® entry clone expression control), Colleclin® Reagent, LR Clonase® enzyme mix, frozen Sf9 insect cells, Grace’s Medium, and ganciclovir.
†† The BaculoDirect™ Transfection Kit includes BaculoDirect™ Linear DNA, Colleclin® Reagent and pENTR™/CAT.

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**Increase microarray sensitivity** with the SuperScript™ Indirect cDNA Labeling System

The SuperScript™ Indirect cDNA Labeling System increases the sensitivity of your microarray experiments. Based on widely used, proven labeling protocols, the system includes SuperScript™ III RT and a unique nucleotide mixture, ensuring better yields and bright signals.

**cDNA yield affects microarray results**

cDNA labeling methods reproduce mRNA populations in isolated samples. To effectively monitor subtle changes in gene expression, it is important to maximize the number of mRNA transcripts that are reverse transcribed into cDNA during the labeling reaction. Full-length cDNAs are optimal because they allow you to analyze internal and 5’ regions of genes of interest. The performance of the reverse transcriptase is the most integral aspect affecting cDNA yield and length. The SuperScript™ Indirect System uses SuperScript™ III RT to consistently generate increased yields (figure 1) and full-length cDNA. This ensures that the labeled cDNA accurately matches your mRNA transcript population and increases the sensitivity of your experiment.

**Signal intensity is important in microarray studies**

Many genes involved in critical cellular pathways are expressed at low to medium abundance levels. Using a sensitive labeling method that provides strong signal intensity enables the detection of low abundance transcripts. The SuperScript™ Indirect System uses a unique amino-allyl/aminohexyl modified nucleotide mixture during cDNA synthesis resulting in efficient and even more incorporation of amino-modified nucleotides for coupling. Labeled cDNA targets hybridize more efficiently and exhibit strong fluorescent signals, increasing sensitivity (figure 2).

**Don’t wait**

There is no reason to compromise on performance. Using the SuperScript™ Indirect cDNA Labeling System, you’ll obtain greater cDNA yields and increased signal intensity, improving the sensitivity of your microarray experiments. Call Invitrogen and order today.

---

**figure 1 - SuperScript™ Indirect cDNA Labeling System generates higher cDNA yields than the competition**

First-strand cDNA was synthesized from 10 µg HeLa total RNA, using the SuperScript™ Indirect cDNA Labeling System and various commercial kits according to manufacturers’ protocols. In each reaction, 32P-α-dCTP was added to trace the cDNA synthesis and 20% of the reaction mixture was spotted on GF/C filters. cDNA yield was calculated according to TCA-precipitated 32P counts.

**figure 2 - SuperScript™ Indirect cDNA Labeling System gives you brighter signals than the competition**

Analysis of arrays (MWG Human Starter) hybridized with Cy3™-labeled cDNA prepared using the SuperScript™ Indirect cDNA Labeling System and competitor kits according to manufacturers’ protocols. Triplicate labeling reactions were performed and hybridized to array. Graph shows the mean signal-to-noise ratios.

---

<table>
<thead>
<tr>
<th>Product</th>
<th>Quantity</th>
<th>Cat. no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>SuperScript™ Indirect cDNA Labeling System</td>
<td>10 rxns</td>
<td>L1014-01</td>
</tr>
<tr>
<td></td>
<td>30 rxns</td>
<td>L1014-02</td>
</tr>
</tbody>
</table>

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Rapid and accurate mammalian two-hybrid analysis

Achieve two-hybrid analysis results in a native mammalian environment faster with the Verve™ Mammalian Two-Hybrid Kit with TOPO® Tools Technology. This unique method replaces time-consuming traditional cloning steps with a 10-minute reaction. You’ll quickly generate bait and prey constructs, saving up to four days of research time.

A better system
Mammalian two-hybrid systems enable the detection and validation of protein-protein interactions in a native environment. Typically, the gene encoding the protein of interest (“bait”) is fused to a DNA binding domain. The gene encoding the potential interacting partner (“prey”) is fused to an activation domain. These constructs are then co-transfected into a mammalian host along with a reporter construct. When the “bait” and “prey” interact, the reporter gene is expressed. Using conventional mammalian two-hybrid methods, you’ll spend days cloning genes to generate the bait and prey constructs. The Verve™ Mammalian Two-Hybrid Kit with TOPO® Tools Technology replaces these time-consuming steps with PCR and a 10-minute TOPO® Joining reaction. You’ll create linear “bait” and “prey” constructs in one day and have results within 24-72 hours.

A faster procedure
Using TOPO® Tools Technology, there are no ligations, no vector manipulations, no cloning, and no E. coli transformations, saving days of time. Simply PCR amplify your “bait” and “prey” genes, join the “bait” product to the P_{SV40/GAL4} 5’ and SV40 pA 3’ elements and the “prey” product to the P_{SV40/VP16} 5’ and SV40 pA 3’ elements in a 10-minute TOPO® Joining reaction, and co-transfect the linear constructs and a reporter plasmid into the mammalian host (figure 1).

Accurately detect interactions
To demonstrate accurate detection of protein interactions, the Verve™ Two-Hybrid Kit and a plasmid-based system were used to detect the known interaction between p53 and the large T antigen in CHO cells. The positive interaction was detected in both systems (figure 2), but results were achieved days earlier using the Verve™ Kit.

Easy high-throughput
Since there are no time-consuming cloning steps, you can easily adapt the Verve™ Two-Hybrid procedure to a high-throughput format and analyze hundreds of interactions.

Speed to two-hybrid analysis
Get faster interaction results with the Verve™ Mammalian Two-Hybrid Kit with TOPO® Tools Technology. Order today.

<table>
<thead>
<tr>
<th>Product</th>
<th>Quantity</th>
<th>Cat. no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verve™ Mammalian Two-Hybrid Kit with TOPO® Tools Technology</td>
<td>100 rxns</td>
<td>T501-100</td>
</tr>
</tbody>
</table>

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The **fastest way** to generate high-level pET expression constructs

**Maximum** protein yields from the Champion™ pET Expression System make it the most powerful system for expressing recombinant proteins. Now cloning into a Champion™ pET vector is faster and more efficient with the Directional TOPO® Cloning and Gateway® Technologies. You’ll get your clone quickly and move on to expression sooner than ever before.

do more with the Champion™ pET vectors

Using the Champion™ pET Expression System, you’ll rapidly produce the highest levels of protein. High-level expression is accomplished via the strong T7lac promoter in the Champion™ pET vectors and a specially designed BL21 strain (BL21 Star™ E. coli, see box). Built for speed, the vectors replace traditional, inefficient restriction enzyme cloning methods with two fast, innovative options:

• **Directional TOPO® Cloning**—clone your gene of interest (goi) in the correct orientation for expression in five minutes with > 90% efficiency

• **Gateway® Technology**—recombine your goi into a pET vector in just one hour; quickly transfer your gene into any number of expression systems without subcloning

You’ll have your expression construct sooner and move on to expression right away.

fast, directional cloning

Champion™ pET Directional TOPO® vectors (table 1) provide rapid, efficient cloning of PCR products using the ligation function of topoisomerase I. Simply add your PCR product directly to the Champion™ pET Directional TOPO® vector, incubate for 5 minutes, and transform into E. coli. That’s it. Greater than 90% of the PCR products will be in the correct 5’ → 3’ orientation. This speed and efficiency means you’ll have your expression clone in just one day.

rapid recombination increases your options

Gateway® Technology* enables you to shuttle your goi into as many vectors and host systems as you choose—without time-consuming subcloning steps. Recombination is fast and efficient, taking just one hour and maintaining directionality. The Champion™ pET Gateway® vectors include att recombination sites so that you can take advantage of this universal cloning and expression platform.

fast cloning leads to speedy results

Generate your T7 expression clone faster and more efficiently using the Champion™ pET Expression System. You’ll get your clone sooner and move on to high-level expression earlier than ever before. Use table 1 below or the information on page 14 to order your Champion™ pET vector today.

**Boost your T7 expression yields**

The BL21 Star™(DE3) One Shot® Chemically Competent cells included in the Champion™ pET Expression System improve your expression yields. BL21 Star™(DE3) E. coli maximize the power of T7 transcription by preventing degradation of transcribed mRNA. This enhanced stability means more mRNA is available for translation, resulting in increased protein production.

* For more information on Gateway® Technology, see the article on pages 14-15 or visit the on-line tutorial at www.invitrogen.com/gateway.

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**table 1 - Champion™ pET vectors adapted with Directional TOPO® Cloning Technology†**

<table>
<thead>
<tr>
<th>Vector</th>
<th>Cat. no.</th>
<th>Position</th>
<th>Tag</th>
<th>Cleavage protease</th>
<th>Antibiotic resistance</th>
<th>Advantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>pET100/D-TOPO®</td>
<td>K100-01</td>
<td>N-term</td>
<td>6xHis-Xpress®</td>
<td>EK</td>
<td>Amp</td>
<td>Cleavable detection and purification tag</td>
</tr>
<tr>
<td>pET101/D-TOPO®</td>
<td>K101-01</td>
<td>C-term</td>
<td>V5-6xHis</td>
<td>–</td>
<td>Amp</td>
<td>Detection and purification tag</td>
</tr>
<tr>
<td>pET102/D-TOPO®</td>
<td>K102-01</td>
<td>N-term</td>
<td>Thioredoxin</td>
<td>EK</td>
<td>Amp</td>
<td>Cleavable thioredoxin tag enhances protein translation and solubility</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Detection and purification tag</td>
</tr>
<tr>
<td>pET151/D-TOPO®</td>
<td>K151-01</td>
<td>N-term</td>
<td>V5-6xHis</td>
<td>TEV</td>
<td>Amp</td>
<td>Cleavable detection and purification tag</td>
</tr>
<tr>
<td>pET200/D-TOPO®</td>
<td>K200-01</td>
<td>N-term</td>
<td>6xHis-Xpress®</td>
<td>EK</td>
<td>Kan</td>
<td>Cleavable detection and purification tag</td>
</tr>
</tbody>
</table>

† Vectors are sold as part of a complete kit that includes linearized, topoisomerase-activated vector, PCR reagents, One Shot® TOP10 E. coli, and BL21 Star™(DE3) One Shot® E. coli.
SimplyBlue™ SafeStain outperforms the competition in protein staining

Allen Bautista, Technical Services, Invitrogen Corporation, Carlsbad, CA

Staining a polyacrylamide gel with Coomassie® blue stain is the most commonly used procedure for visualizing protein bands. Traditional Coomassie® stains are slow and do not offer the sensitivity that many experiments require. SimplyBlue™ SafeStain is a uniquely formulated, ready-to-use, colloidal Coomassie® G-250 stain that is specifically designed to give fast, sensitive staining results. Here we demonstrate that SimplyBlue™ yields results five-times faster with at least twice the sensitivity of other commonly used Coomassie® staining reagents.

methods

Gel electrophoresis. Three NuPAGE® Novex 4-12% Bis-Tris Gels were identically loaded with different amounts of various protein samples. The proteins were separated using MES SDS Running Buffer in an XCell SureLock™ Mini-Cell.

Staining. After electrophoresis, two of the three gels were washed two times, five minutes each, with 100 ml of ultrapure water. One of these gels was stained with 20 ml of SimplyBlue™ SafeStain. The other was stained with 20 ml of GelCode® Blue (Pierce Chemical Company). Both gels were stained for one hour. The stains were then decanted and the gels were destained in 100 ml of ultrapure water for one hour. After one hour, 20 ml of a 20% NaCl solution was added to the water of the gel stained with SimplyBlue™ SafeStain. The third gel was stained with 20 ml of the conventional Coomassie® R-250 stain (0.1% Brilliant blue R-250 in 50% ethanol, 10% acetic acid) for 30 minutes. This gel was then destained in 10% acetic acid for 30 minutes. Band development, background, and sensitivity for each gel was analyzed with scanned images taken at various time points in the protocol.

results

Staining time. The results demonstrate that with SimplyBlue™ SafeStain, proteins stain five-times faster than with other Coomassie® stains (table 1 and figure 1, page 7). After 5 minutes in the SimplyBlue™ SafeStain, 1 µg of protein is detected (Gel A, lanes 2 and 3). After 5 minutes in the GelCode® Blue stain, 6 µg of protein is only faintly detected (Gel B, lane 1). Using GelCode® Blue, the 1 µg bands are only visible after 25 minutes in the stain (image not shown). Although 1 µg of protein can be detected after 5 minutes in the conventional Coomassie® R-250 stain (Gel C, lanes 2 and 3), background is high, obscuring the detection of lower concentrations of protein and the Mark12™ Unstained Standard (Gel C, lanes 9 and 10).

Sensitivity. SimplyBlue™ SafeStain is at least two times more sensitive than other Coomassie® stains (table 1, figure 1, page 7). After two hours in the water-based destain, 7 ng of reduced BSA can be detected on the gel stained with SimplyBlue™ SafeStain (Gel D, lane 7). Only 20 ng of reduced BSA can be detected on the gels stained with either GelCode® Blue or conventional R-250 stain. When left overnight in the water-based destain, as little as 3 ng of reduced BSA can be detected on the gel stained with SimplyBlue™ SafeStain (Gel G, lane 8). When the GelCode® Blue-stained gel is left overnight in water destain, only 20 ng can be detected (Gel H, lane 5). Slightly increased sensitivity is seen on the gel stained with conventional R-250 stain (Gel I, lane 6).

<table>
<thead>
<tr>
<th>Speed</th>
<th>SimplyBlue™ SafeStain</th>
<th>GelCode® Blue</th>
<th>Coomassie® R-250</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 minutes in stain</td>
<td>1 µg BSA</td>
<td>6 µg BSA</td>
<td>1 µg BSA</td>
<td>Mark12™ and low concentrations of BSA were detected with SimplyBlue™ SafeStain. High background with R-250 stain.</td>
</tr>
<tr>
<td>2 hours in recommended destaining solution*</td>
<td>7 ng BSA</td>
<td>20 ng BSA</td>
<td>20 ng BSA</td>
<td>SimplyBlue™ SafeStain demonstrates the highest sensitivity.</td>
</tr>
<tr>
<td>Overnight in recommended destaining solution*</td>
<td>3 ng BSA</td>
<td>20 ng BSA</td>
<td>&gt; 10 ng BSA</td>
<td>No loss in sensitivity when gels are kept overnight in SimplyBlue™ water-based destain.</td>
</tr>
</tbody>
</table>

*Destain for SimplyBlue™ consists of 20% NaCl in water. Destain for GelCode® Blue consists of water as recommended by manufacturer. Destain for R-250 Coomassie stain consists of 10% acetic acid in water.

continued on page 7
SimplyBlue™ SafeStain provides faster, more sensitive staining results than other Coomassie® stains. It allows identification of protein bands quickly and without ambiguity, enabling further analysis to proceed promptly. Achieve the maximum-performance protein staining results your experiments require. Call Invitrogen and order SimplyBlue™ SafeStain today.

*Sufficient reagent is supplied to stain 50 mini-gels.

**Results in 12 minutes with the SimplyBlue™ SafeStain microwave procedure**

Achieve ultra-fast Coomassie® staining results with the SimplyBlue™ SafeStain microwave procedure. Detect as little as 20 ng† in 12 minutes. See the protocol pull-out card in this issue for details.

† 20 ng reduced BSA

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**discussion**

The following samples were electrophoresed on NuPAGE® Novex 4-12% Bis-Tris Gels and then stained with SimplyBlue™ SafeStain, GelCode® Blue, or Coomassie® R-250 following manufacturer-recommended protocols. Items to note are listed below each gel. Arrows indicate achieved sensitivity.

<table>
<thead>
<tr>
<th>Lane</th>
<th>Description</th>
<th>Quantity</th>
<th>Cat. no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6 µg protein mix</td>
<td>1 L*</td>
<td>LC6060</td>
</tr>
<tr>
<td>2</td>
<td>1 µg rabbit IgG</td>
<td>3.5 L</td>
<td>LC6065</td>
</tr>
<tr>
<td>3</td>
<td>1 µg reduced BSA</td>
<td>1 L*</td>
<td>LC6060</td>
</tr>
<tr>
<td>4</td>
<td>5 µg E. coli lysate</td>
<td>1 L*</td>
<td>LC6060</td>
</tr>
<tr>
<td>5</td>
<td>20 ng reduced BSA</td>
<td>1 L*</td>
<td>LC6060</td>
</tr>
<tr>
<td>6</td>
<td>10 ng reduced BSA</td>
<td>1 L*</td>
<td>LC6060</td>
</tr>
<tr>
<td>7</td>
<td>7 ng reduced BSA</td>
<td>1 L*</td>
<td>LC6060</td>
</tr>
<tr>
<td>8</td>
<td>3 ng reduced BSA</td>
<td>1 L*</td>
<td>LC6060</td>
</tr>
<tr>
<td>9</td>
<td>10 µl Mark12™ Standard</td>
<td>1 L*</td>
<td>LC6060</td>
</tr>
<tr>
<td>10</td>
<td>5 µl Mark12™ Standard</td>
<td>1 L*</td>
<td>LC6060</td>
</tr>
</tbody>
</table>

---

**figure 1 - comparison of speed and staining sensitivity of three Coomassie® stains**

---

<table>
<thead>
<tr>
<th>SimplyBlue™ SafeStain</th>
<th>GelCode® Blue</th>
<th>Coomassie® R-250 Stain</th>
</tr>
</thead>
<tbody>
<tr>
<td>After 5 minutes in stain</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Lane 1: 6 µg protein mix</td>
<td>• Sensitivity: 1 µg protein</td>
<td>• Sensitivity: 6 µg protein</td>
</tr>
<tr>
<td>Lane 2: 1 µg rabbit IgG</td>
<td>• Mark12™ clearly visible</td>
<td>• Mark12™ not visible</td>
</tr>
<tr>
<td>Lane 3: 1 µg reduced BSA</td>
<td>• Water-based destain</td>
<td>• Water destain</td>
</tr>
<tr>
<td>After 2 hours in destain</td>
<td>D</td>
<td>E</td>
</tr>
<tr>
<td>Lane 4: 5 µg E. coli lysate</td>
<td>• Water-based destain</td>
<td>• Water destain</td>
</tr>
<tr>
<td>Lane 5: 20 ng reduced BSA</td>
<td>• Sensitivity: 7 ng reduced BSA</td>
<td>• Sensitivity: 20 ng reduced BSA</td>
</tr>
<tr>
<td>Lane 6: 10 ng reduced BSA</td>
<td>• No loss in sensitivity with overnight water-based destaining</td>
<td>• Loss in sensitivity with overnight water destaining</td>
</tr>
<tr>
<td>After overnight in destain</td>
<td>G</td>
<td>H</td>
</tr>
<tr>
<td>Lane 7: 7 ng reduced BSA</td>
<td>• Water-based destain</td>
<td>• Water destain</td>
</tr>
<tr>
<td>Lane 8: 3 ng reduced BSA</td>
<td>• Sensitivity: 57 ng reduced BSA</td>
<td>• Sensitivity: 20 ng reduced BSA</td>
</tr>
<tr>
<td>Lane 9: 10 µl Mark12™ Standard</td>
<td>• Mark12™ clearly visible</td>
<td>• Mark12™ not visible</td>
</tr>
<tr>
<td>Lane 10: 5 µl Mark12™ Standard</td>
<td>• Sensitivity: 1 µg protein</td>
<td>• Sensitivity: 1 µg protein</td>
</tr>
</tbody>
</table>
Clone genes **faster and more efficiently** with two new competent cells for cloning

Two new *E. coli* strains increase the speed and improve the efficiency of cloning new genes. Mach1™ T1 Phage-Resistant (T1R) chemically competent *E. coli* is the fastest growing cloning strain on the market, saving you valuable research time. OmniMAX™ T1 Phage-Resistant (T1R) chemically competent *E. coli* provide the highest transformation efficiencies. With these two strains, you’ll clone genes faster and more efficiently than ever before.

**new cloning strains**

Until now, competent cells failed to address two cloning bottlenecks: slow-growing bacteria and high-efficiency transformations in a convenient format. Not any more. Mach1™ T1R cells grow rapidly, allowing you to plate and pick colonies the same day or to perform mini-preps from a four-hour culture. OmniMAX™ T1 R *E. coli* is an improved DH5α™ strain that combines versatile genetic markers with high transformation efficiency, enabling you to get your clone first time.

**fastest growing strain**

Think of how much faster you could clone genes if you didn’t wait on overnight bacterial cultures or could plate and pick bacterial colonies the same day. With the fastest-growing cloning strain, Mach1™ T1R *E. coli*, the wait is over. Sufficient growth for mini-preps is achieved from an overnight colony in just four hours compared to overnight for most other cloning strains, saving you an entire day (figure 1).

**most competent strain**

OmniMAX™ T1 Phage-resistant *E. coli* provide transformation efficiencies > 5 x 10⁹ cfu/µg, giving you a better chance of getting your desired clone (figure 2). In addition, these cells lack methylation restriction systems, which means you can transform both methylated and non-methylated DNA. Being resistant to both T1 and T5 phages means your cultures are safe from phage contamination and your valuable samples are protected. Use OmniMAX™-T1R cells for unsurpassed results in your cloning experiments.

**give them a shot**

Mach1™-T1R *E. coli* offers you significant savings in time due to its fast growth rate. OmniMAX™-T1R *E. coli* is an improved DH5α™ strain that combines all the important genetic elements for cloning with the highest transformation efficiencies available. Use these new competent cells to transform the way you clone today.

---

**figure 1 - time saved using Mach1™-T1R E. coli**

<table>
<thead>
<tr>
<th>Competent Cells</th>
<th>Time to mini-prep</th>
<th>Time to colony formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mach1™-T1R</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>Other cloning strains</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

* With ampicillin selection.

**figure 2 - OmniMAX™-T1R cells provide superior transformation efficiency**

Transformation efficiency of One Shot® OmniMAX™-T1R, XL10-Gold®, and MAX Efficiency® DH5α™ cells was determined using 10 pg pUC19 plasmid. Transformations were performed according to manufacturer’s protocols. Values represented are mean ± SD of triplicate observations.

**Product** | Efficiency | Quantity | Cat. no. |
---|---|---|---|
One Shot® Mach1™-T1R Chemically Competent *E. coli* | > 1 x 10⁹ | 20 x 50 µl | C8620-03 |
MultiShot® StripWell Mach1™-T1R Chemically Competent *E. coli* | > 1 x 10⁹ | 1 plate | C8696-01 |
One Shot® OmniMAX™-T1R Chemically Competent *E. coli* | > 5 x 10⁹ | 20 x 50 µl | C8520-03 |
MultiShot® StripWell OmniMAX™-T1R Chemically Competent *E. coli* | > 1 x 10⁹ | 1 plate | C8596-01 |

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High-performance one-step RT-PCR for end point and real-time applications

Get the superior yield of SuperScript™ III Reverse Transcriptase and the hot-start specificity of Platinum® Taq DNA Polymerase in two new one-step RT-PCR kits. Choose the SuperScript™ III One-Step RT-PCR System with Platinum® Taq for sensitive end point analysis or the SuperScript™ III Platinum® One-Step qRT-PCR Kit for high-performance real-time detection.

proven enzymes for great results
Both the SuperScript™ III One-Step RT-PCR System and the SuperScript™ III Platinum® One-Step qRT-PCR Kit combine SuperScript™ III RT and Platinum® Taq to give you exceptionally sensitive and specific one-step RT-PCR. SuperScript™ III RT is a RNase H- mutant of SuperScript™ II RT that exhibits a longer half-life (220 min. at 50°C) and increased thermostability, giving you higher cDNA yields, greater success with RNA secondary structure, and increased specificity with gene-specific primers. Platinum® Taq DNA Polymerase provides hot-start technology to reduce mispriming for greater specificity and sensitivity in your PCR. Whether you’re doing end point or real-time detection, these proven enzymes guarantee great performance.

sensitive end point analysis
The SuperScript™ III One-Step RT-PCR System with Platinum® Taq is optimized for sensitive end point analysis. The 2X reaction buffer and enzyme mix have been improved to provide increased sensitivity—down to 0.01 pg total RNA, the most sensitive system available (figure 1).

optimized real-time detection
The SuperScript™ III Platinum® One-Step qRT-PCR Kit has been designed and optimized to deliver the best real-time RT-PCR results. A specially formulated buffer system produces superior performance across multiple instrument platforms, and provides you with the flexibility to detect with LUX™ Fluorogenic Primers* (figure 2) or dual-labeled probes without comprising results. In addition, larger kit sizes provide more reactions to accommodate high-throughput needs, streamlining your real-time experiments.

taking one-step farther
Take your one-step RT-PCR farther with your choice of high-performance kits specifically optimized for end point or real-time analysis. Call Invitrogen and order the SuperScript™ III One-Step RT-PCR System or the SuperScript™ III Platinum® One-Step qRT-PCR Kit today.

* For more information on LUX™ Fluorogenic Primers, please visit www.invitrogen.com/lux.

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**figure 1** - the SuperScript™ III One-Step RT-PCR System with Platinum® Taq provides superior sensitivity

One-Step RT-PCR reactions containing 0.01, 0.1, and 1 pg of total HeLa RNA were performed using the SuperScript™ III One-Step RT-PCR System with Platinum® Taq DNA Polymerase. RT incubation was 55°C for 30 minutes, followed by 40 cycles of PCR, 1 min/kb.

**figure 2** - the SuperScript™ III Platinum® One-Step qRT-PCR Kit is optimized for real-time detection

The human β-actin transcript was quantified by one-step quantitative RT-PCR from 10-fold serial dilutions of human HeLa RNA (100 ng to 0.1 pg) using 200 nM JOE-labeled LUX™ Primer, 200 nM unlabeled primer, SuperScript™ III Platinum® One-Step qRT-PCR kit, and ROX Reference Dye. The resulting standard curve equation was: $y = -3.410x + 44.166$. $R^2 = 0.998$. 

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<table>
<thead>
<tr>
<th>Product</th>
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<tr>
<td>SuperScript™ III One-Step RT-PCR System with Platinum® Taq</td>
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<td>12574-018</td>
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<tr>
<td></td>
<td>100 rxns</td>
<td>12574-026</td>
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<td>SuperScript™ III Platinum® One-Step qRT-PCR Kit</td>
<td>100 rxns</td>
<td>11732-020</td>
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<td></td>
<td>500 rxns</td>
<td>11732-088</td>
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</table>

Visit www.invitrogen.com for more information.
**Flexible protein expression**—get native or tagged protein on demand

Producing both native and epitope-tagged protein for different applications currently requires subcloning your gene into two different vectors. Now you can eliminate additional subcloning steps with Tag-On-Demand™ Technology. Express native or tagged recombinant protein from one vector, so you can easily and quickly get to your downstream studies.

**tag it or don’t, as you please**
Detection of expressed proteins is often facilitated by the use of epitope tags. This is particularly helpful when a protein-specific antibody for your recombinant protein is not available. However, for some experimental approaches it is important to express native protein. In the past, expressing your recombinant protein in both epitope-tagged and native forms required subcloning your gene twice and maintaining two vector stocks. With the state-of-the-art Tag-On-Demand™ Technology, you only have to clone your gene once to easily express your recombinant protein with or without a tag. You’ll get native protein expressed until you add the Tag-On-Demand™ Suppressor Supernatant. Then you’ll produce high levels of your protein tagged with GFP or V5. It’s easy to choose how you want your protein expressed with Tag-On-Demand™ Technology. You’ll save time and effort too, by avoiding those extra subcloning steps.

**high-performance vectors**
There are currently two mammalian Tag-On-Demand™ expression vectors available, pcDNA™6.2/V5-DEST and pcDNA™6.2/GFP-DEST (figure 1), that allow you to take advantage of the Tag-On-Demand™ Technology. Each vector offers:

- A C-terminal V5 or GFP epitope tag for efficient protein detection—when added to your protein if the open reading frame (ORF) uses an amber (TAG) stop codon that can be suppressed
- **att** sites for use in Gateway® Technology to easily transfer your gene into other host systems or vectors
- CMV promoter for high-level expression

In addition, each vector includes the Blasticidin resistance gene for rapid and efficient selection of stable cell lines. Choose the V5 antibody epitope tag for extremely sensitive detection by western blotting or the GFP tag for easy visual detection in live cells.

**how it works**
The key to the Tag-On-Demand™ system is the Suppressor Supernatant. This recombinant adenoviral supernatant delivers a suppressor tRNA gene that recognizes the TAG, or amber, stop codon present at the end of your ORF. Simply add the Tag-On-Demand™ Supernatant directly to cells that contain a Tag-On-Demand™ expression vector to produce tagged protein (figures 2 and 3, page 11). In the absence of the Tag-On-Demand™ Supernatant, the TAG stop codon is recognized and you’ll express native protein. Now you can easily detect your protein via epitope antibody and conduct subcellular localization studies via immunostaining or fluorescence when you want, or produce fully native protein from the same expression vector.

**efficient suppression**
Upon the addition of the Tag-On-Demand™ Suppressor Supernatant, you’ll get efficient and specific suppression of TAG stop codons, resulting in greater than 50% of your recombinant protein tagged with your choice of V5 or GFP (figure 4, page 11). Using a Tag-On-Demand™ DEST™ vector, you’ll get high levels of protein expression and efficient tagging when you want, and native protein when you don’t.

**ultimate convenience and compatibility**
The Tag-On-Demand™ vector kits are conveniently packaged with either the

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continued on page 11
PCDNA™6.2/V5-DEST or PCDNA™6.2/GFP-DEST vector and Tag-On-Demand™ Suppressor Supernatant. Use any Gateway® entry clone that has your protein expressed with a TAG stop. Tag-On-Demand™ Technology is also fully compatible with any of the ready-to-use Ultimate™ Human ORF Clones provided as sequence-verified Gateway® entry clones.

Add a small aliquot of Tag-On-Demand™ Suppressor Supernatant at the time of transfection for expression of epitope-tagged protein. Your gene of interest (GOI) with a TAG stop codon should be recombined into a pcDNA™6.2/V5-DEST or pcDNA™6.2/GFP-DEST vector from any Gateway® entry clone or any Ultimate™ ORF Clone. The GOI will be placed under the control of the CMV promoter and in-frame with a downstream V5 or GFP epitope that will be fused to the C-terminus of the protein. The epitope tag is followed by all three stop codons. In the absence of the Tag-On-Demand™ Suppressor Supernatant, native protein is expressed following transient transfection with the expression vector. If the Tag-On-Demand™ Suppressor Supernatant is added, most of the protein will express the epitope tag 48 hours post-transfection due to read through of the TAG stop codon and placement of a serine residue at this codon.

CHO cells were co-transfected with a pcDNA™3.2/V5-GW/CATTAG construct using Lipofectamine™ 2000 Reagent, and protein was expressed in the absence (-) or presence (+) of the Tag-On-Demand™ Suppressor Supernatant. Forty-eight hours post-transfection, 20 µg of cell lysate was analyzed using an Anti-CAT antibody on a western blot. This shows that in the presence of the Tag-On-Demand™ Suppressor Supernatant, most of the expressed protein has been shifted up due to the presence of the V5 tag.
**Speed up your research with the ready-to-go Ultimate™ Mouse ORF Clones**

The Ultimate™ ORF Clone Collection consists of full-length, full-insert sequenced open reading frames (ORFs) in a Gateway® entry vector. This collection now includes mouse ORFs. Using the Ultimate™ Mouse ORF Clones, you can quickly begin studying the effects of your gene of interest without tedious preparation steps and limiting single-system vectors.

**time-saving mouse resource**

The Ultimate™ Mouse ORF Clone Collection consists of ready-to-use clones built specifically for functional analysis. Each Ultimate™ ORF Clone includes only the gene sequence, without a specific promoter, in the pENTR™221 Gateway® entry vector for unlimited downstream analysis options. Stringent quality control procedures ensure the clone identity and sequence validity. There's no need for you to spend your time isolating RNA, synthesizing cDNA, cloning, and verifying sequence, saving weeks of time and effort. Just purchase your clone(s) of interest and start your downstream studies immediately.

**easily enter any system**

With Gateway® Technology, you can transfer your gene of interest into different systems and hosts for analysis. A simple one-hour recombination reaction maintains directionality and sequence fidelity, eliminating time-consuming subcloning and revalidation steps. The Ultimate™ Mouse ORF Clones are provided in the pENTR™221 Gateway® entry vector (figure 1), allowing you to take advantage of this state-of-the-art technology. From this vector you can transfer your gene of interest into a comprehensive selection of Gateway® destination vectors for expression in cell-free, E. coli, yeast, insect, or mammalian systems.

**to tag or not to tag**

In addition to choosing your host, the Ultimate™ ORF Clones allow you to express your gene from a single vector with or without a C-terminal tag.* Each gene coding sequence is followed by an amber stop codon. During translation, this stop codon is recognized and you’ll express native protein. Adding the specially formulated Tag-On-Demand™ Suppressor Supernatant to the expression culture suppresses this stop codon and produces a protein fused to the downstream vector elements*.

**stringent requirements**

Before you receive an Ultimate™ Mouse ORF Clone, it undergoes a series of quality control tests. All clones are full-insert sequenced and the amino acid sequence is guaranteed to match the corresponding sequence in GenBank. Additionally, clone identity is confirmed before shipment. You can be confident that the clone you request is the clone you receive.

**find your clone on-line today**

Many Ultimate™ Mouse ORF Clones are currently available. To find your clone, visit www.invitrogen.com/clones. In just a few clicks you can order the clone you need today and start your functional analysis studies quicker than ever before.

<table>
<thead>
<tr>
<th>Product</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Ultimate™ Mouse ORF Clones</td>
<td>MORF01</td>
</tr>
</tbody>
</table>

* Applies only when recombined into a Tag-On-Demand™ vector. To learn more about Tag-On-Demand™ Technology, check out the article on page 10 of this issue of Expressions.

**Expression 10.4 1 800 955 6288**
Easy on-line ordering of custom antibodies and peptides

Now it’s easier than ever to order high-quality EvoQuest™ custom peptides and antibodies. Three new on-line ordering forms—for custom peptide synthesis, custom peptide-antibody production, and antibody production with user-supplied immunogen—take the guesswork out of your project.

at your fingertips
Ordering peptides and antibodies takes extensive planning and consideration. What scale and purity do I use for my peptide? Which conjugation strategy is the best to use? What animal species do I use to raise antibodies? To get the most out of your project, you don’t want to miss any aspect. The new on-line ordering forms from EvoQuest™ Custom Services walk you through the ordering process, so you can be confident that all your options are presented.

ordering made simple
Using the on-line ordering forms is straightforward. You can access the two antibody ordering forms (user-supplied immunogen or peptide synthesis and antibody production) on the custom antibody web site at www.invitrogen.com/polyclonal. The peptide form is located at www.invitrogen.com/customs. All you need to do is to register, and a username and password will be given to you. The forms will then guide you through the ordering process step by step (figure 1).

no more guessing
Each custom on-line ordering form takes you through the numerous options for peptide and antibody production. Easy-to-use drop down menus provide lists of available choices (figure 1). You’ll also find a price sheet with a brief description of services, instructions on how to enter the peptide sequence with modifications, and directions for ordering peptide design.

try it out
The three on-line ordering forms for custom antibody and peptide services take the guesswork out of ordering. Try them today at www.invitrogen.com/polyclonal or call EvoQuest™ customer service at 800 955 6288 ext. 45682.

Invitrogen acquires PanVera—more resources, more innovation

The powerful new partnership of Invitrogen and PanVera gives you a dependable single resource for a broad spectrum of product lines to help you attain your drug discovery research goals more simply, more effectively, and more efficiently.

high-quality, dependable biochemical assays and purified proteins
You can identify potential targets more rapidly and precisely with PanVera’s robust Kinase, Nuclear Receptor, and Drug Metabolism assays and purified proteins. The newest of these products include the:

- reliable, easy-to-use Z'LYTE™ Kinase and Phosphatase Assays
- sensitive, reliable Terbium Chelate Labeling Products for detecting intermolecular interactions
- dependable, sensitive Vivid® P450 Screening Kits for drug metabolism research

sensitive cell-based assays
Two highly accurate technologies are available to meet your cell-based assay needs:

- Beta-Lactamase Assays (BLA) for measuring gene expression in mammalian cells
- Voltage Sensor Probes (VSPs) for ion channel research to rapidly detect any target that changes the membrane potential of a cell

a synergistic new relationship
You now have the combined resources of two companies to help further your research. Visit our web sites (www.panvera.com or www.invitrogen.com) for more information.

www.invitrogen.com Expressions 10.4
**Comprehensive selection** of Gateway® expression vectors to meet your research needs

Gateway® Technology allows you to clone your gene of interest into an entry vector and then shuttle it—without traditional subcloning—into a variety of expression vectors, saving time and accelerating discovery. A large selection of expression vectors compatible with the Gateway® Technology is available, so no matter what your downstream analysis needs are, you can get there more efficiently.

**unlimited expression options**
Successful expression of a given gene from a particular promoter, host, or system is not guaranteed. To get the results you need, you may have to use a variety of vectors and systems. With the Gateway® Technology, you can access a wide variety of expression and functional analysis systems without additional time-consuming subcloning and sequencing steps.

**fast entry into Gateway® Technology**
Access to unlimited downstream capabilities is rapid and efficient following generation of a Gateway® entry clone. Simply clone your gene of interest into a Gateway® entry vector. Whether you prefer TOPO® Cloning, PCR Cloning, or restriction digestion and ligation, an option is available for you. Or simply purchase a pre-made, sequence-validated open reading frame (ORF) that is already cloned into an entry vector by ordering from the Ultimate® ORF Clone Collection.*

**easy to use**
Once you have your gene of interest or ORF in a Gateway® entry vector, select a Gateway® destination vector from the wide selection of available (table 1). You can quickly and easily shuttle your gene of interest from the entry clone into one or more destination vectors. Simply incubate the entry clone and destination vector in a Gateway® recombination reaction for one hour at room temperature. Then transform into *E. coli* and plate. It’s that easy.

**try Gateway® today**
No matter which system you choose, you will get results faster with the flexibility of Gateway® Technology. Find out more about Gateway® at www.invitrogen.com/gateway.

* For a list of clones available in the Ultimate® ORF Clone Collection, visit www.invitrogen.com/clones.

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**table 1** - a wide variety of Gateway® destination vectors are available to meet your downstream analysis needs

<table>
<thead>
<tr>
<th>Vector</th>
<th>Advantages</th>
<th>Cat. no.</th>
</tr>
</thead>
</table>
| pEXP1-DEST™ | • T7 promoter, ribosome-binding site, and T7 terminator for efficient protein synthesis  
• N-terminal Xpress™ tag for convenient detection of fusion proteins; tag can be removed for native expression  
• N-terminal 6xHis sequence for rapid purification | V960-01 |
| pEXP2-DEST™ | • T7 promoter and T7 terminator for efficient expression  
• C-terminal 6xHis tag for easy detection and rapid purification | V960-02 |

**Expression in *E. coli***

| Champion™ pET-DEST™42 | • T7 promoter for high yields of recombinant protein  
• LacO operator sequences for lac repressor binding for tighter regulation  
• Lac repressor for tight regulation of transcription in *E. coli*  
• C-terminal 6xHis tag for easy detection and rapid purification | 12276-010 |
| Champion™ pET104-DEST™ | • T7 promoter for high yields of recombinant protein  
• N-terminal BioEase™ tag for in vivo biotinylation of fusion proteins | R104-01 |
| pDEST™14, pDEST™15, pDEST™24 | • T7 promoter under control of the T7 RNA polymerase for high-level expression  
• No tag for native protein expression (pDEST™14)  
• GST tag for easy column purification, pDEST™15 (N-terminal) and pDEST™24 (C-terminal)  
• N-terminal 6xHis tag for rapid purification (pDEST™17) | 11801-016, 11802-014, 12216-016, 11803-012 |

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continued on page 15
### Gateway® Technology

**continued from page 14**

<table>
<thead>
<tr>
<th>Vector</th>
<th>Advantages</th>
<th>Cat. no.</th>
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</thead>
</table>
| **pBAD-DEST™49** | • araBAD promoter for tightly regulated expression of toxic proteins  
• N-terminal thioredoxin fusion partner for efficient protein translation and improved solubility  
• C-terminal V5-6xHis tag for easy detection and rapid purification | 12283-016 |

**Optimized, inducible expression in *S. cerevisiae***

<table>
<thead>
<tr>
<th>Vector</th>
<th>Advantages</th>
<th>Cat. no.</th>
</tr>
</thead>
</table>
| **pYES-DEST™52** | • GAL7 promoter for high-level, inducible expression in *S. cerevisiae*  
• C-terminal V5-6xHis tag for easy detection and rapid purification | 12286-019 |

**Expression in insect cells**

<table>
<thead>
<tr>
<th>Vector</th>
<th>Advantages</th>
<th>Cat. no.</th>
</tr>
</thead>
</table>
| **BaculoDirect™ DNA** | • BaculoDirect™ Linear DNA for easiest method of baculovirus production, ideal for HTP  
• Thymidine kinase (TK) gene for negative selection of non-recombinant baculovirus  
• LacZ gene to determine viral stock purity  
• C-terminal V5-6xHis tag for easy detection and rapid purification | 12562-013 |
| **pDEST™8** | • Bac-to-Bac® vectors for rapid baculovirus production for small scale expression  
• No tag for native expression (pDEST™8)  
• N-terminal 6xHis tag for rapid purification (pDEST™8)  
• N-terminal GST tag for easy column purification (pDEST™20) | 11804-010, 11805-015, 11807-013 |
| **pMT-DEST™48** | • DES® vector, an easy-to-use system for constitutive or inducible expression  
• Metallothionein promoter for high-level, metal-inducible expression in *Drosophila* S2 cells  
• C-terminal V5-6xHis tag for easy detection and rapid purification | 12282-018 |
| **pMT/BioEase™-DEST** | • Metallothionein promoter for high-level, metal-inducible expression in *Drosophila* S2 cells  
• N-terminal BioEase™ tag for in vivo biotinylation of fusion proteins | V4140-20 |

**Expression in mammalian cells**

<table>
<thead>
<tr>
<th>Vector</th>
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</table>
| **pcDNA™-DEST40** | • CMV promoter for high-level expression mammalian cells and Geneticin® marker for robust selection (except pcDNA™6.2/V5-DEST)  
• C-terminal 6xHis tag for easy detection and rapid purification (pcDNA™-DEST40) | 12274-015 |
| **pcDNA™-DEST47, pcDNA™-DEST53** | • GFP tag for rapid fluorescent detection of recombinant protein (pcDNA™-DEST47 (C-terminal)) and pcDNA™-DEST53 (N-terminal))  
• C-terminal V5 tag for easy detection (pcDNA™-DEST47 and pcDNA™-6.2/V5-DEST)  
• Blasticidin resistance gene for efficient stable selection (pcDNA™-6.2/V5-DEST)  
• N-terminal 6xHis tag for easy detection (pcDNA™-3.1/nV5-DEST)  
• N-terminal 6xHis tag for rapid purification (pDEST™26)  
• N-terminal GST tag for easy column purification (pDEST™27) | 12281-010, 12288-015, 12249-019, 12249-017, 12290-010, 11809-019, 11812-013 |
| **pT-REx-DEST™30** | • CMV/TetO promoter for tetracycline-regulated expression and highest levels of induced expression  
• No tag for native protein expression (pT-REx-DEST™30)  
• N-terminal 6xHis tag for rapid purification (pT-REx-DEST™31) | 12301-016, 12302-014 |
| **pK/F-R/F/Y5-DEST™** | • EF-1α promoter (for high-level constitutive expression from a non-viral promoter)  
• C-terminal V5-6xHis tag for easy detection and rapid purification  
• Flp recombinase target (FRT) site for efficient integration into Flp-In™ Cell Lines  
• Hygromycin resistance gene for convenient selection of integrants  
• C-terminal V5 tag for easy detection | 12285-011, V6020-20 |

**Viral delivery systems**

<table>
<thead>
<tr>
<th>Vector</th>
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<th>Cat. no.</th>
</tr>
</thead>
</table>
| **plent6/V5-DEST™** | • CMV promoter for high-level expression in a variety of mammalian cells  
• VSV-G envelope protein for transduction in a broad host range  
• C-terminal V5 tag for easy detection  
• Choice of either Blasticidin (plent6/V5-DEST™) or Zeocin® (plent4/V5-DEST™) resistance genes for efficient selection and option to generate double stable cell lines | V496-10, V498-10 |
| **plent6/UbC/V5-DEST™** | • Human ubiquitin promoter (UbC) for in vivo studies in animal models or for physiological expression levels in mammalian cells  
• C-terminal V5 tag for easy detection | V499-10 |
| **pAd/CMV/V5-DEST™** | • CMV promoter for high-level expression in a variety of mammalian cells (pAd/CMV/V5-DEST™) or Promoterless vector for flexibility to use your own promoter (pAd/PL-DEST™)  
• E1 and E3 genes deleted for production of replication-incompetent virus  
• C-terminal V5 tag for easy detection | V493-20, V494-20 |
Ready-to-use western blotting kits provide faster protein detection

Most commercially available western blotting detection systems require you to spend a substantial amount of time optimizing reagents and protocols. The pre-optimized WesternBreeze® Immunodetection Kits eliminate tedious optimization steps and allow you to quickly and easily obtain western blot results. You’ll save time and effort on every western blot.

How can WesternBreeze® Immunodetection Kits speed up my protein detection?

WesternBreeze® is the only complete, pre-optimized western blotting system designed to obtain sensitive results in minimal time. Unlike other blotting kits, each WesternBreeze® Kit includes all the reagents you need for blotting and detection with your own primary antibody. All kit reagents have been optimized at a predetermined concentration and tested to work together to ensure great results. There’s no need for you to spend time and effort gathering, preparing, and testing your own solutions. With WesternBreeze®, all of the optimization work has been done for you, saving you hours of time and effort.

How much time can I save with WesternBreeze®?

The fast and simple WesternBreeze® protocol allows you to get great western blot results in less than three hours. The time required for all major steps—including blocking, primary antibody and secondary antibody incubation—is much less than that needed in conventional protocols (table 1). In addition, the WesternBreeze® ready-to-use and easy-to-dilute reagents minimize the time spent preparing solutions. All together, you’ll save up to 17 hours with WesternBreeze® Kits over other western blot detection methods.

How can I visualize the signals achieved with WesternBreeze®?

The alkaline phosphatase (AP)-based WesternBreeze® Immunodetection Kits enable you to easily visualize your protein bands using either chemiluminescent light emission or colorimetric stain. The WesternBreeze® Chemiluminescent Kits apply a ready-to-use CDP-Star® substrate that is catalyzed by AP to produce light signals at the specific protein bands. These signals can be captured by X-ray film (figure 1A), CCD-camera image systems, and most luminometers. The WesternBreeze® Chromogenic Kits use a ready-to-use BCIP/NBT* substrate that is catalyzed by AP to form a stable purple color at the specific bands directly on the membrane (figure 1B). With WesternBreeze®, you’ll be able to easily obtain western blot results by film, image system, or colorimetric stain, whichever is suitable for your laboratory setting.

<table>
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<th>Major Steps</th>
<th>WesternBreeze® Protocol</th>
<th>Conventional Protocol</th>
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<tr>
<td>Prepare solutions</td>
<td>5 minutes</td>
<td>30 minutes</td>
</tr>
<tr>
<td>Incubate membrane in blocking solution</td>
<td>30 minutes</td>
<td>1 to 15 hours</td>
</tr>
<tr>
<td>Incubate membrane in primary antibody</td>
<td>1 hour</td>
<td>1 to 2 hours</td>
</tr>
<tr>
<td>Incubate membrane in secondary antibody</td>
<td>30 minutes</td>
<td>1 to 2 hours</td>
</tr>
<tr>
<td>Total time</td>
<td>&lt; 3 hours</td>
<td>4 to 20 hours</td>
</tr>
</tbody>
</table>

fast and easy protein detection

You can save time and effort in both optimizing solutions and obtaining final results with the WesternBreeze® Immunodetection Kits. Six kits are currently available so you can choose the one that best fits your needs. To experience fast protein detection with minimal effort, call Invitrogen and place your order today.

<table>
<thead>
<tr>
<th>Product</th>
<th>Quantity†</th>
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<td>Anti-Mouse 1 kit</td>
<td>WB7103</td>
<td>$205</td>
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<tr>
<td></td>
<td>Anti-Rabbit 1 kit</td>
<td>WB7105</td>
<td>$205</td>
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<tr>
<td></td>
<td>Anti-Goat 1 kit</td>
<td>WB7107</td>
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<tr>
<td>WesternBreeze® Chemiluminescent Detection Kit</td>
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<td>Anti-Rabbit 1 kit</td>
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<td></td>
<td>Anti-Goat 1 kit</td>
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<td>$255</td>
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</table>

* BCIP/NBT is a compound of 5-bromo-4-chloro-3-indolyl-1-phosphate/nitro blue tetrazolium.
† Each kit supplies sufficient reagents for detection of 20 mini-blots.
Improved PCR SuperMixes offer quality and convenience in one

You save valuable time when you use PCR SuperMixes because all of the reagents you need for PCR are pre-mixed. Now you will also have increased peace of mind because the improved quality control procedures guarantee higher consistency.

**convenience**

PCR is an extremely useful tool that is often more time consuming than many users would like. Invitrogen PCR SuperMixes are the ideal answer to this dilemma. These pre-mixed solutions save you valuable time by combining all of the PCR components—enzyme, hot-start antibodies, dNTPs, magnesium, and buffers—in one vial. Strict quality control guidelines ensure product consistency to improve your PCR results. With PCR SuperMixes, you’ll get the convenience and reliable PCR performance you need.

**high-quality reagents**

PCR SuperMix and Platinum® PCR SuperMix are designed for reliability and high quality. To ensure that you continue to achieve reliable results, the quality control procedures for these products are now even more rigorous. Functional testing is done on multiple vials in every lot of each SuperMix manufactured. Band quality, consistency, and intensity are visualized on an agarose gel from the PCR done in triplicate. Tests are also done to confirm the absence of contaminants such as DNase, RNase, and exonuclease. As a result of this extensive quality control testing, you will get reliable PCR performance.

**consistency and peace of mind**

PCR SuperMix and Platinum® PCR SuperMix consistently amplify targets up to 5 kb, with very little variability between lots (figure 1). You can have complete confidence that these two high-performance SuperMixes produce you reliable results the first time, every time.

**selection to meet your PCR needs**

PCR SuperMixes are available in multiple formats for use in all of your PCR applications. PCR SuperMix is for routine PCR, offering the cost savings of recombinant *Taq* DNA Polymerase with the convenience of a SuperMix. Platinum® PCR SuperMix includes Platinum® hot-start antibodies for improved PCR specificity and yield as well as the convenience of room temperature reaction set-up. Additional PCR SuperMixes are available to meet all of your PCR needs. These include Platinum® PCR SuperMix 96—all the benefits of the original Platinum® PCR SuperMix in a 96-well format. Call or visit the Invitrogen web site for information on additional PCR SuperMixes.

**order today**

For reliable, convenient PCR performance, choose PCR SuperMix and Platinum® PCR SuperMix. Order one today.

<table>
<thead>
<tr>
<th>Product</th>
<th>Quantity</th>
<th>Cat. no.</th>
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<tbody>
<tr>
<td>PCR SuperMix</td>
<td>100 reactions</td>
<td>10572-014</td>
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<td></td>
<td>5000 reactions</td>
<td>10572-063</td>
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<tr>
<td>Platinum® PCR SuperMix</td>
<td>100 reactions</td>
<td>11306-016</td>
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<tr>
<td></td>
<td>5000 reactions</td>
<td>11306-081</td>
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<tr>
<td>Platinum® PCR SuperMix 96</td>
<td>skirted 5 x 96-well plates</td>
<td>11306-065</td>
</tr>
<tr>
<td></td>
<td>nonskirted 5 x 96-well plates</td>
<td>11306-073</td>
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Make active protein in a scalable, two-hour cell-free reaction

The Expressway™ Plus Expression System utilizes an efficient coupled transcription and translation reaction to produce active recombinant protein. In just two hours, you can produce up to milligrams of protein suitable for an array of downstream applications.

the cell-free advantage

*In vitro*, cell-free expression systems take less time and eliminate several of the time-consuming steps required by cell-based expression systems such as transformation, transfection, and maintenance of cell cultures. Although there are significant advantages to using a cell-free expression system, these methods often produce extremely low amounts of protein. With the Expressway™ Plus System, you can scale your expression to suit your downstream needs. The reaction is scalable from micrograms to milligrams of protein depending upon the reaction size. Whichever you choose, you’ll have the protein yield you need in a simple two-hour reaction.

active protein made easy

The Expressway™ Plus System is a simple-to-use, cell-free expression system. Simply combine the reaction buffer, methionine, and T7 RNA polymerase with the highly active *E. coli* extract. Then add your DNA template containing a T7 promoter, ribosome binding site (RBS), and your gene of interest. The reaction-driving *E. coli* extract quickly transcribes and translates active protein from the DNA template (figure 1). The Expressway™ Plus reaction requires only two hours to complete, saving you hours of time over traditional expression methods.

scalable reaction

To demonstrate the scalability of the Expressway™ Plus System, reaction volumes ranging from 50 µl to 10 ml were compared. Following a two-hour incubation, protein yields from each reaction were also determined (figure 2). Increasing the reaction volume resulted in a subsequent increase in protein yield. Using the Expressway™ Plus System, you can produce milligrams of proteins and get to your downstream experiments faster.

more protein, fast and easy

Speed up your protein production and get your results faster with the Expressway™ Plus Expression System. Call and order today.

<table>
<thead>
<tr>
<th>Product</th>
<th>Quantity</th>
<th>Cat. no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expressway™ Plus Expression System*</td>
<td>20 reactions</td>
<td>K9900-01</td>
</tr>
<tr>
<td>with pEXP1-DEST™</td>
<td>20 reactions</td>
<td>K9900-02</td>
</tr>
<tr>
<td>with pEXP2-DEST™</td>
<td>20 reactions</td>
<td>K9900-03</td>
</tr>
</tbody>
</table>

* Each kit includes IVPS Plus *E. coli* Extract, 2.5X IVPS Plus *E. coli* Reaction Buffer, RNase A, T7 Enzyme Mix, Methionine, reaction tubes, control plasmids, and the appropriate expression vector when indicated.

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**Expand your expression profiling studies**

The I-SAGE™ Long Kit improves the widely referenced serial analysis of gene expression (SAGE™) method, enabling more accurate EST database comparisons and direct mapping of tags to genomic location. The I-SAGE™ Long Kit is an ideal complement to microarray analysis or an excellent alternative for researchers seeking an effective method of expression profiling without the limitations of microarray technology.

---

### Improved expression profiling

Though powerful in their ability to simultaneously monitor thousands of differentially expressed genes, microarrays limit expression profiling to the number of probes attached to their surface and require gene sequence knowledge. Moreover, variations in sample preparation, slide preparation, hybridization, and normalization techniques make data comparisons between microarray experiments difficult. SAGE™ circumvents these limitations, allowing you to monitor gene expression of every transcript in a cell, generate quantitative data, and effectively detect low abundance transcripts. The new I-SAGE™ Long Kit offers all the benefits of SAGE™ and more. Using an improved protocol, the I-SAGE™ Long Kit generates a longer tag than is possible with the original SAGE™ methods, allowing clearer discrimination between genes and improving novel gene isolation.

### Increased accuracy

The original SAGE™ method isolates a 14-bp tag from each mRNA transcript expressed in a sample. Tags are ligated, cloned, and sequenced in high throughput. Raw sequence data is analyzed using SAGE™ data analysis software, producing digital results detailing the presence and corresponding frequency of mRNA transcripts (1). The I-SAGE™ Long Kit uses a similar protocol, but with a new restriction endonuclease, MMEI, producing a 21-bp tag. The additional tag length enhances the identification of mRNA transcripts, enabling more accurate comparisons to reference EST databases (2).

### Enhance downstream studies

The additional length of Long SAGE™ tags provides sufficient complexity to accurately map tags directly to their genomic location, enabling you to match differentially expressed transcripts to their chromosomal location (table 1). You’ll be able correlate increases or decreases in gene expression with biological function and identify disease related genes. After comparing your I-SAGE™ Long libraries to reference EST databases in SAGEmap or SAGE Genie*, you can use unique Long SAGE™ tags to design PCR primers for use in Rapid Amplification of cDNA ends (RACE) applications or as probes to screen cDNA libraries, facilitating novel gene isolation (2).

---

### Complete kit saves time

Sourcing and optimizing the nearly 50 reagents necessary to create Long SAGE™ libraries is not necessary. The I-SAGE™ Long Kit contains all the required reagents to successfully generate Long SAGE™ libraries in one functionally tested kit.

### Improve your SAGE™ studies today

The I-SAGE™ Long Kit enables you to enjoy the benefits of Long SAGE™ and improve your expression profiling. Call and order today.

---

### Table 1 - Theoretical Matching of Tags to Genome

<table>
<thead>
<tr>
<th>Tag length (n base pairs)</th>
<th>Complexity</th>
<th>Tag uniqueness probability†</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>1,048,576</td>
<td>0.00%</td>
</tr>
<tr>
<td>15</td>
<td>4,194,304</td>
<td>0.08%</td>
</tr>
<tr>
<td>16</td>
<td>16,777,216</td>
<td>16.73%</td>
</tr>
<tr>
<td>18</td>
<td>67,108,864</td>
<td>63.95%</td>
</tr>
<tr>
<td>19</td>
<td>1,073,741,824</td>
<td>97.24%</td>
</tr>
<tr>
<td>20</td>
<td>4,294,967,296</td>
<td>99.30%</td>
</tr>
<tr>
<td>21</td>
<td>17,179,869,184</td>
<td>99.83%</td>
</tr>
</tbody>
</table>

* Complexity of tags (C=4^n) is determined using a tag length comprising a constant 4 bp representing the restriction site at which the transcript was cleaved, followed by n bp derived from the adjacent sequence in each transcript.

† The probability that a tag is unique in the genome (P_u=[C-1/C]^n) is determined under the assumption that the genome contains ~30 x 10⁶ Nla III-derived tags and is comprised of random sequence.

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### Product Table

<table>
<thead>
<tr>
<th>Product</th>
<th>Quantity</th>
<th>Cat. no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>I-SAGE™ Long Kit</td>
<td>5 Libraries</td>
<td>T5000-03</td>
</tr>
<tr>
<td>I-SAGE™ Long Ditag PCR Module</td>
<td>1,000 PCR rxns</td>
<td>T5000-04</td>
</tr>
<tr>
<td>Invitrogen™ Magnetic Stand</td>
<td>1</td>
<td>R670-01</td>
</tr>
</tbody>
</table>


### References:


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Increase microarray sensitivity . . . . . .3
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Clone genes faster and more efficiently . . . 8
SuperScript™ III One-Step RT-PCR Systems for End Point and Real-time Analysis
High-performance one-step RT-PCR for end point and real-time applications . . 9
Tag-On-Demand™ Technology
Flexible protein expression—get native or tagged protein on demand . . . . . .10
Ultimate™ Mouse ORF Clones
Speed up your research with ready-to-go clones . . . . . . . .12
Expressway™ Plus Expression System
Make active protein in a scalable, two-hour cell-free reaction . . . . . . . .18
I-SAGE™ Long Kit
Expand your expression profiling studies . . . . . . . .19

application note

SimplyBlue™ SafeStain
SimplyBlue™ SafeStain outperforms the competition in protein staining . . . . . .6

product reviews

Verve™ Mammalian Two-Hybrid Kit with TOPO® Tools Technology
Rapid and accurate mammalian two-hybrid analysis . . . . . . . . . . . . . .4

Champion™ pET Expression System
The fastest way to generate high-level pET expression constructs . . . . . . . .5
EvoQuest™ Custom Peptides and Antibodies
Easy on-line ordering . . . . . . . . . . . . . . . . . . . . . . . . . . . .13
Gateway® Technology
Comprehensive selection of Gateway® expression vectors . . . . . . . . . . .14
PCR SuperMix
Improved PCR SuperMixes offer quality and convenience in one . . . . . . .17

announcement

Invitrogen acquires PanVera . . . . . . . . .13

Q&A

WesternBreeze® Immunodetection Kits
Ready-to-use western blotting kits provide faster protein detection . . . . . . .16

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