July 2005

EasyXpress™ Insect Cell Protein Synthesis Handbook

EasyXpress Protein Synthesis Insect Kit

EasyXpress pIX4.0 Vector

For in vitro synthesis of proteins with posttranslational modifications using insect-cell lysates

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Contents

Kit Contents

EasyXpress Protein Synthesis Insect Kit For 20 x 50 µl reactions	**Cat. no. 32552**
EasyXpress Insect Extract (colorless snap-cap)	4 x 100 µl
EasyXpress Insect Reaction Buffer (blue screw-cap)	2 x 50 µl
EasyXpress Insect Energy Mix (red screw-cap)	2 x 50 µl
RNase-Free Water (colorless screw-cap)	1 x 1.9 ml
EasyXpress Insect Positive Control DNA (violet screw-cap)	1 x 25 µl
5x Transcription Buffer (yellow screw-cap)	1 x 100 µl
5x NTP Mix (green screw-cap)	4 x 25 µl
20x Enzyme Mix (orange screw-cap)	1 x 25 µl
DyeEx® 2.0 Spin Columns	20
Collection Tubes (2 ml)	20
Handbook	1

EasyXpress pIX4.0 Vector	**Cat. no. 32713**
EasyXpress pIX4.0 Vector (white screw-cap)	25 µg (0.5 µg/µl)
Handbook	1

Storage and Stability

The EasyXpress Protein Synthesis Insect Kit is shipped on dry ice.

5x Transcription Buffer, **5x NTP Mix**, and **20x Enzyme Mix** must be stored at –20°C upon arrival.

EasyXpress Insect Extract, **EasyXpress Insect Reaction Buffer**, and

EasyXpress Insect Positive Control DNA must be stored at –70°C upon arrival.
Once thawed, EasyXpress Insect extract should be stored on ice and used within 4 hours.

The **EasyXpress pIX4.0 Vector** is shipped on dry ice and must be stored at –20°C upon arrival.

DyeEx 2.0 Spin Columns should be stored dry at room temperature (15–25°C). For longer storage, these kits can be stored at 2–8°C. Do not freeze.

When stored under the above conditions and handled correctly, the EasyXpress Protein Synthesis Insect Kit can be stored for at least 6 months without showing any reduction in performance. The EasyXpress pIX4.0 Vector can be stored for at least 1 year.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online in convenient and compact PDF format at www.qiagen.com/ts/msds.asp where you can find, view, and print the MSDS for each QIAGEN kit and kit component.

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany, Tel: +49-6131-19240

Quality Control

In accordance with QIAGEN's ISO-certified Total Quality Management System, each lot of EasyXpress Kits is tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations

EasyXpress kits are developed, designed, and sold for research purposes only. It is not to be used for human diagnostic or drug purposes or to be administered to humans unless expressly cleared for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. All due care and attention should be exercised in the handling of many of the materials described in this text.

Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN product does not meet your expectations, simply call your local Technical Service Department or distributor. We will credit your account or exchange the product — as you wish.

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor (see inside back cover).

Technical Assistance

At QIAGEN we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of QIAGEN® products. If you have any questions or experience any difficulties regarding EasyXpress kits or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information please call one of the QIAGEN Technical Service Departments or local distributors (see inside back cover).

Introduction

In vitro translation is a widely used tool for the production of recombinant proteins. Proteins produced by in vitro translation can be used for a wide variety of downstream applications, including activity assays, protein–protein interaction studies, and the expression and analysis of open reading frames and expression constructs.

A broad range of eukaryotic proteins require posttranslational modifications such as phosphorylation, glycosylation, or signal peptide cleavage to display full functional activity. Eukaryotic in vitro translation systems provide the possibility to synthesize eukaryotic proteins with posttranslational modifications and are of special importance for expression and analysis of human proteins with native structure and function.

To address these needs, QIAGEN has developed the EasyXpress Protein Synthesis Insect Kit, a new eukaryotic in vitro translation system for expression of eukaryotic proteins — including membrane proteins — with posttranslational modifications. In contrast to many rabbit-reticulocyte lysate (RRL) -based systems, the insect-cell extract does not require any additives to display full functionality. In addition, the EasyXpress pIX4.0 Vector has been developed for generation of optimal expression templates to be used with the EasyXpress Protein Synthesis Insect Kit.

The EasyXpress Insect Protein Synthesis System

The EasyXpress Protein Synthesis Insect Kit uses highly productive insect cell lysates obtained from a *Spodoptera frugiperda* cell line, which contain all translational machinery components (i.e., ribosomes, ribosomal factors, tRNAs, aminoacyl-tRNA synthetases, etc.) required for efficient protein synthesis. In addition, the lysates contain functional organellar membrane fractions, whose activity is required for posttranslational modification of eukaryotic proteins, including membrane proteins (Figures 1 and 2). It is a linked transcription–translation system (see flowchart, page 9).

In the in vitro transcription reaction, high-quality capped mRNA is produced using linearized plasmid DNA containing a T7 promoter. In the linked in vitro translation reaction, the capped mRNA is used as template to express active full-length proteins.

Using the EasyXpress Insect Kit, up to 15 µg/ml functionally active posttranslationally modified protein can be synthesized within 3.5 hours (1).

Efficient Synthesis of a Wide Range of Proteins

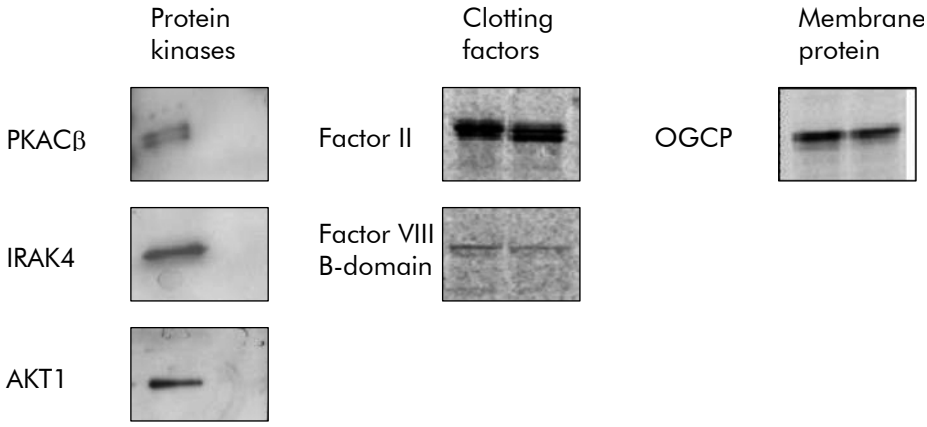


Figure 1 Proteins successfully expressed using the EasyXpress Protein Synthesis Insect Kit. 6xHis-tagged protein kinases were visualized using the Penta·His HRP Conjugate. Clotting factors and OGCP were synthesized in duplicate reactions using ^{14}C-labeled amino acids and visualized using a PhosphorImager®.

Efficient Synthesis of Glycosylated Proteins Using Insect Cell Extracts

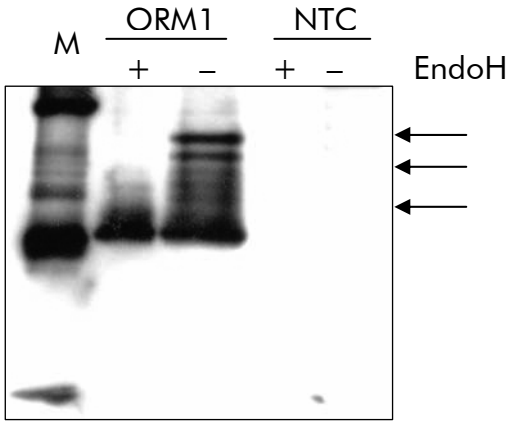
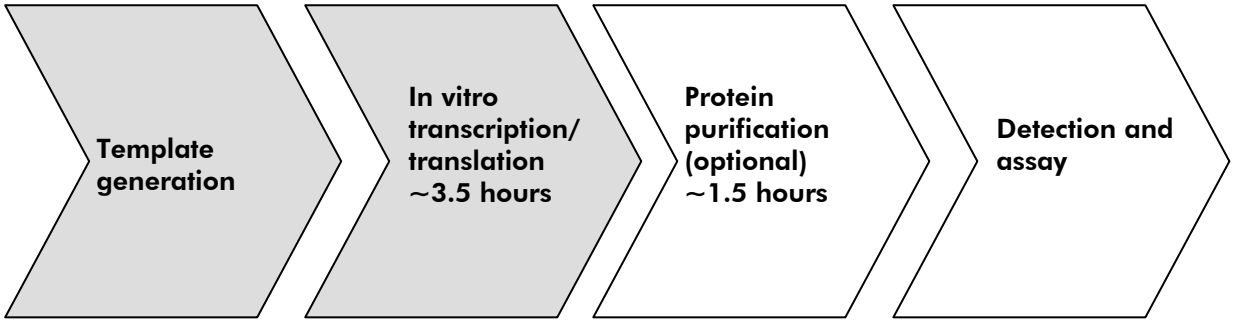


Figure 2 The glycoprotein ORM1 (human alpha-1-acid glycoprotein 1) was synthesized as a 6xHis-tagged construct using the EasyXpress Protein Synthesis Insect Kit. After purification, aliquots of the synthesis reactions were then incubated in the presence or absence of the endoglycosidase EndoH, which cleaves glycan moieties from glycoproteins. After separation by SDS-PAGE and western blotting, proteins were visualized using the Penta·His Antibody. Removal of the glycan moieties by EndoH treatment increases the electrophoretic mobility of the protein compared to the glycosylated forms (arrowed). **NTC**: no template control; **M**: markers (6xHis Protein Ladder).

Workflow for In Vitro Protein Synthesis Using the EasyXpress Insect System and Downstream Purification, Detection, and Assay



EasyXpress pIX4.0 Vector

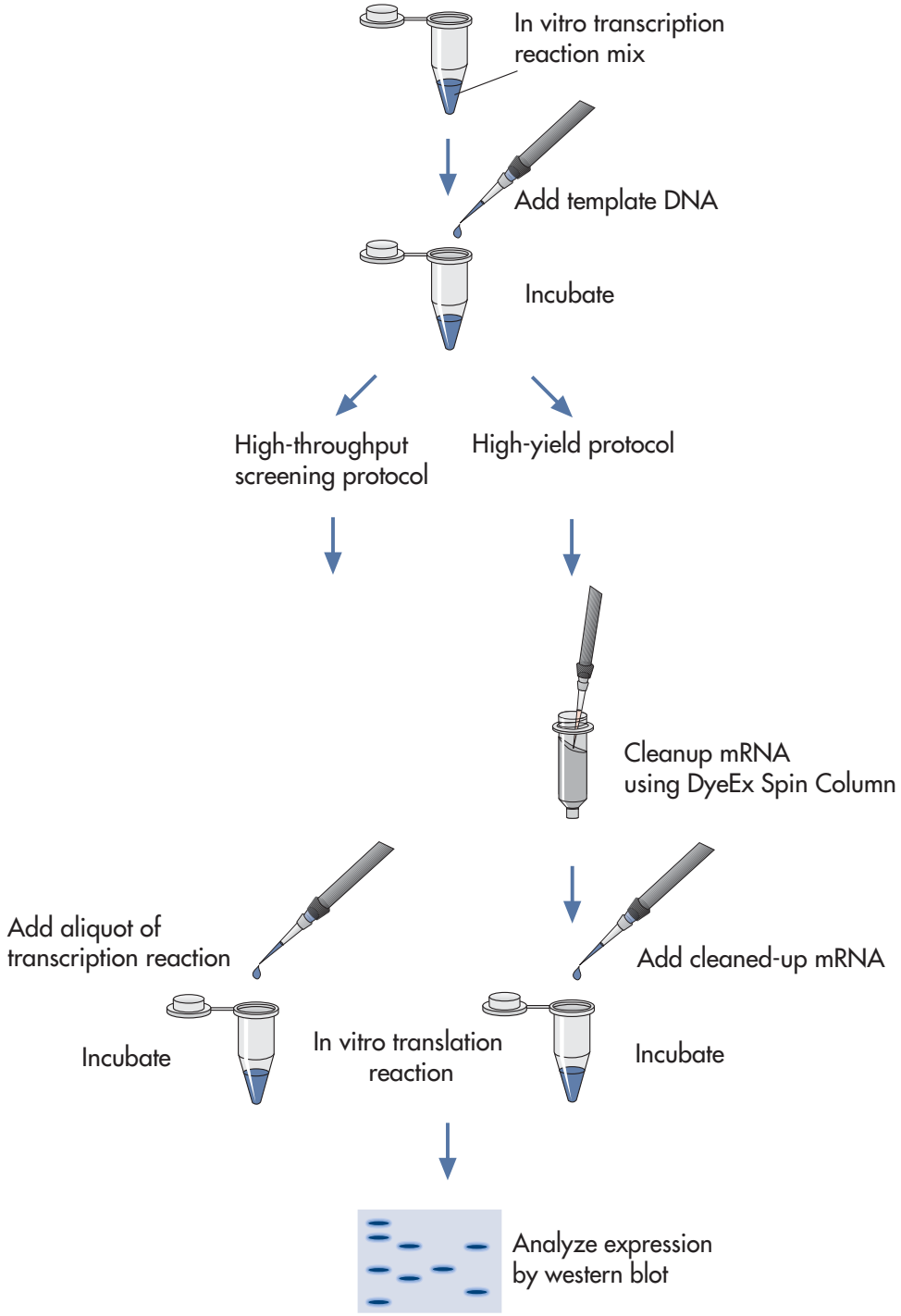
EasyXpress Protein Synthesis Insect Kit

Ni-NTA Magnetic Agarose Beads

Penta·His Antibody or Penta·His HRP Conjugate Kit

Strep-tag Antibody

EasyXpress Protein Synthesis Procedure



DNA Templates

The EasyXpress Protein Synthesis Insect Kit can be used to express proteins from a variety of DNA templates. Templates must contain a T7 promoter upstream of the coding sequence. Suitable DNA templates include linearized plasmid DNA.

Minimum template requirements

DNA templates must contain the T7 promoter (Figure 3) for transcription. A stretch of at least 5 base pairs should be placed upstream of the promoter. The sequence of the transcribed mRNA must begin with at least one G. The 5' untranslated region (5'-UTR) must not contain an ATG triplet in any reading frame. Strong secondary structures within the 5'-UTR should be avoided. The translation start codon must be ATG and the translation stop codon must be TAA, TAG, or TGA.

For optimal efficiency of transcription and translation we strongly recommend using the cloning and expression vector EasyXpress pIX4.0 (cat. no. 32713), see Figure 4, page 13.

5'...XXXXX**TAATACGACTCACTATA**G... 3'

Figure 3 Sequence of T7 promoter (bold) and transcription start (underlined).

Plasmid DNA

Greatest yields of capped mRNA resulting in high protein yields are obtained using template DNA of the highest purity. High-purity plasmid DNA can easily be obtained with the QIAGEN HiSpeed®, QIAfilter, and QIAprep® Kits. DNA prepared using the standard alkaline lysis method described by Sambrook, Fritsch, and Maniatis (2) may be sufficiently pure, but DNA must be free of RNases.

Prior to in vitro transcription, the plasmid DNA must be linearized using a restriction enzyme that cuts downstream of the insert to be transcribed. We recommend using restriction enzymes that produce blunt ends. Restriction enzymes that produce 3' overhangs should be avoided.

Following digestion, linearized DNA should be cleaned up, for example using the QIAquick® Nucleotide Removal Kit (QIAGEN, cat. no. 28304) and resuspended in RNase-free water.

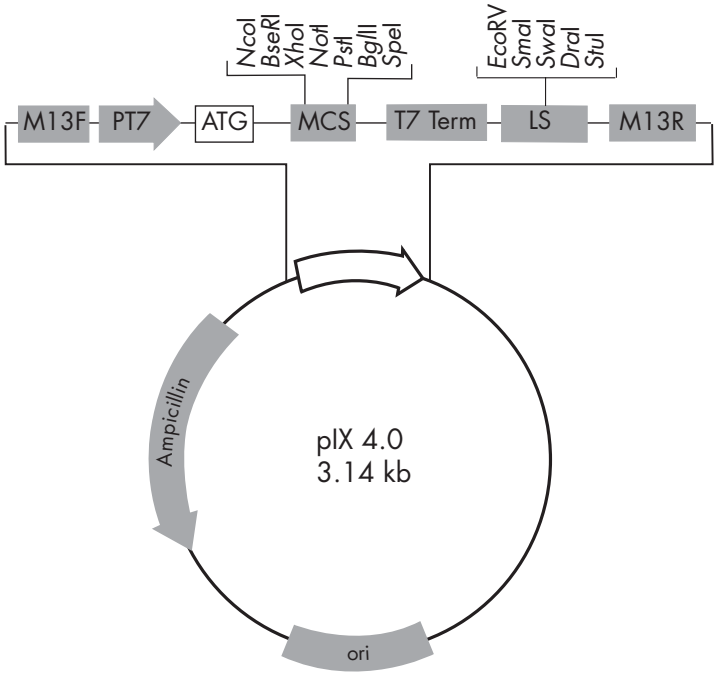
For linearized DNA encoding mRNAs of less than 1500 bases, 1 µg linearized DNA template should be added to each 25 µl in vitro transcription reaction at a concentration of 0.2 µg/µl. In some cases, the yield of protein synthesis can be improved by increasing the DNA amount to 1.5 µg or decreasing the amount to 0.5 µg per 25 µl transcription reaction. Alternatively, the volume of cleaned up mRNA (see protocol on page 19) added to the translation reaction can be lowered to 6 µl or increased to 20 µl.

For linearized DNA encoding mRNAs of greater than 1500 bases, 1.5 µg linearized DNA template should be added to each 25 µl in vitro transcription reaction at a concentration of 0.3 µg/µl. In some cases, the yield of protein synthesis can be improved by increasing the DNA amount to 2.0 µg or decreasing the amount to 1.0 µg per 25 µl transcription reaction. Alternatively, the volume of cleaned up mRNA (see protocol on page 19) added to the translation reaction can be lowered to 6 µl or increased to 20 µl.

EasyXpress pIX4.0 Vector

The EasyXpress pIX4.0 Vector is designed for high-level expression of proteins using the EasyXpress Protein Synthesis Insect Kit. This high-copy plasmid has the following features:

- Efficient initiation of translation through a T7 promoter element combined with an optimized 3' UTR, a T7 terminator, and an optimally positioned linearization site
- Multiple cloning site and translational stop codons in all reading frames for convenient preparation of expression constructs
- Optimized 3' UTR combined with T7 terminator for generation of stabilized RNA protected from degradation by exonucleolytic nucleases
- Site for plasmid linearization consisting of multiple restriction sites for blunt end linearization for effective in vitro transcription
- β-lactamase gene conferring resistance to ampicillin



*Nco*I *Bse*RI *Xho*I *Not*I *Pst*I *Bgl*II *Spe*I

CC**ATG**GGAGACCCCTCCTCGAGCGGCCGCCTGCAGATCTAAATAATAAGTAATTAACTAGT
GGTACCCTCTGGGGAGGAGCTCGCCGGCGGACGTCTAGATTTATTATTCATTAATTGATCA
Met Gly Asp Pro Ser Ser Ser Gly Arg Leu Gln Ile

Figure 4 The pIX4.0 vector. **M13F**: M13 forward, **PT7**: T7 promoter, **MCS**: multiple cloning site, **T7 term**: T7 terminator, **LS**: Linearization site, **M13R:** M13 reverse, **ori**: origin of replication.

Designing Primers for Cloning into the EasyXpress pIX4.0 Vector

The choice of primers and cloning procedure depends on the desired N- and C-terminal structure/sequence. The possibilities include:

- Protein sequence starting with an N-terminus Met followed by the amino acid Gly, Glu, Asp, Ala, or Val. See "Cloning into pIX4.0 via *Nco*I", Appendix E, page 35.
- Proteins that are synthesized free of any additional vector amino acids. See "Cloning into pIX4.0 via *Bse*RI", Appendix E, page 38.
- Protein contains up to 6 vector-encoded N-terminal amino acids. See "Cloning into pIX4.0 via *Xho*I, *Not*I, *Pst*I, *Bgl*II", Appendix E, page 41.
- Protein is synthesized with an N-terminal 6xHis or *Strep*-tag®. See "PCR-mediated addition of affinity-tag sequences", Appendix E, page 44.
- Protein is synthesized with a C-terminal 6xHis or *Strep*-tag. See "PCR-mediated addition of affinity-tag sequences", Appendix E, page 44.
- Protein is synthesized with a 6xHis or *Strep*-tag at both the N- and C-terminus. See "PCR-mediated addition of affinity-tag sequences", Appendix E, page 44.

Purification of In Vitro-Synthesized Proteins Carrying a 6xHis tag

In vitro-synthesized proteins that carry a 6xHis tag can be easily purified using Ni-NTA Magnetic Beads. For a purification protocol, see Appendix A, page 24.

His-tagged protein purification is based on the remarkable selectivity and high affinity of patented Ni-NTA (nickel-nitrilotriacetic acid) resin for proteins containing an affinity tag of six consecutive histidine residues — the 6xHis tag. NTA, which has four chelation sites for nickel ions, binds nickel more tightly than metal-chelating purification systems that only have three sites available for interaction with metal ions. The extra chelation site prevents nickel-ion leaching; providing a greater binding capacity, and high-purity protein preparations.

Protocol: Protein Synthesis Using the EasyXpress Protein Synthesis Insect Kit

This protocol is suitable for the in vitro synthesis of recombinant proteins with posttranslational modifications using the EasyXpress Protein Synthesis Insect Kit.

The protocol is divided into two sections, in vitro transcription and in vitro translation. A fast high-throughput version and a high-yield version of the translation protocol are provided. Using the positive control DNA template, the high-throughput and high-yield protocols deliver up to 6 µg and up to 15 µg active luciferase per ml reaction respectively.

Materials and reagents to be supplied by the user

- Linearized plasmid DNA encoding the protein of interest. The plasmid must contain a T7 promoter (see page 11)
- Thermomixer (Eppendorf, Hamburg, Germany)

Important points before starting

- The in vitro translation system is extremely sensitive to nuclease contamination. Always wear gloves and use RNase- and DNase-free reaction tubes and filter pipet tips.
- EasyXpress Insect Extract is provided as 4 individual aliquots in single tubes. Once thawed, store EasyXpress Insect extract on ice and use within 4 hours. Do not refreeze and thaw more than four times. Refreeze the extracts in liquid nitrogen.
- The recommended incubation temperature for transcription is 37°C, the incubation temperature for protein synthesis is 27°C.

Procedure

In vitro transcription reaction

1. **Thaw and store 5x Transcription Buffer, 5x NTP Mix, 20x Enzyme Mix, RNase-Free Water, and EasyXpress Insect Positive Control DNA on ice.**
2. **Pipet together the components of the three transcription reactions shown in Table 1 in three DNase- and RNase-free 1.5 ml microcentrifuge tubes at room temperature (15–25°C). Label each tube clearly.**

   It is important to add the reaction components in the order shown in Table 1.

Table 1. Pipetting Scheme for Transcription Reactions

Reagent	Target protein template reaction	Positive control reaction	No template control reaction
RNase-Free water	8.75 µl	8.75 µl	13.75 µl
5x Transcription Buffer	5 µl	5 µl	5 µl
5x NTP Mix	5 µl	5 µl	5 µl
20x Enzyme Mix	1.25 µl	1.25 µl	1.25 µl
EasyXpress Insect Positive Control DNA	—	5 µl	—
Linearized DNA or PCR product	5 µl*	—	—
Total	25 µl	25 µl	25 µl

* For linearized DNA encoding mRNAs of less than 1500 bases, 1 µg linearized DNA template should be added to each 25 µl in vitro transcription reaction at a concentration of 0.2 µg/µl. For linearized DNA encoding mRNAs of greater than 1500 bases, 1.5 µg linearized DNA template should be added to each 25 µl in vitro transcription reaction at a concentration of 0.3 µg/µl.

3. **Mix all components by vortexing and briefly centrifuge to collect the reactions at the bottom of the tubes.**
4. **Incubate the in vitro transcription reactions for 120 min at 37°C.**
5. **Centrifuge the reactions for 1 min at 12,000 x *g* at room temperature (15–25°C).**
6. **For the high-throughput screening protocol proceed from step 7a. For the high-yield protocol proceed from 7b on page 19.**

In vitro translation reaction using the high-throughput screening protocol

7a. Without further processing, use 5 µl of each in vitro transcription reaction for an in vitro translation reaction.

8a. Thaw and store EasyXpress Insect Reaction Buffer, EasyXpress Insect Extract, EasyXpress Energy Mix, and RNase-Free Water on ice.

9a. Pipet together the components of the three translation reactions shown in Table 2 in three DNase- and RNase-free 1.5 ml microcentrifuge tubes. Label each tube clearly.

It is important to add the reaction components in the order shown in Table 2.

Table 2. Pipetting Scheme for High-Throughput Screening In Vitro Translation Reactions

Reagent	Target protein synthesis reaction	Positive control reaction	No template control reaction
RNase-Free Water	15 µl	15 µl	15 µl
EasyXpress Insect Reaction Buffer	5 µl	5 µl	5 µl
EasyXpress Insect Extract	20 µl	20 µl	20 µl
Template mRNA*	5 µl	5 µl	5 µl
Insect EasyXpress Energy Mix	5 µl	5 µl	5 µl
Total	**50 µl**	**50 µl**	**50 µl**

* From step 5. Use an aliquot of the respective transcription reaction.

10a. Mix all components by vortexing and briefly centrifuge to collect the reactions at the bottom of the tubes.

11a. Incubate the in vitro translation reactions in a Thermomixer for 90 min at 27°C and 500 rpm.

In vitro translation reaction using the high-yield protocol

In this protocol, mRNA is cleaned up using a DyeEx gel-filtration spin column before addition to the translation reaction.

Important note before starting

All centrifugation steps are performed at 750 x g in a conventional microcentrifuge. The appropriate speed for individual centrifuges can be calculated as follows: rpm = 1000 x √(750/1.12 r) (r = radius of rotor in mm).

Table 3. Examples of suitable microcentrifuges and the corresponding speeds

Microcentrifuge	Speed
Eppendorf® Centrifuge 5415C	3000 rpm
Eppendorf Centrifuge 5417C	2700 rpm
Heraeus Biofuge 15	2800 rpm
Hettich Mikro 24-48	2630 rpm
Beckman GS15R	2100 rpm
Hettich Mikro EBA12	2700 rpm

Procedure

7b. Gently vortex the DyeEx spin column to resuspend the resin.

8b. Loosen the cap of the column a quarter turn.

This is necessary to avoid a vacuum developing inside the spin column.

9b. Snap off the bottom closure of the spin column (Figure 5), and place the spin column in a 2 ml collection tube (provided).

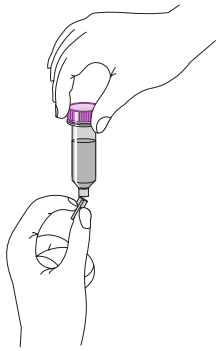


Figure 5 Snapping off the bottom closure of the DyeEx 2.0 spin column (do not screw).

10b. Centrifuge for 3 min at the calculated speed (see Table 3).

11b. Carefully transfer the spin column to a clean centrifuge tube. Slowly apply the 20 µl in vitro transcription reaction to the gel bed (Figure 6).

Pipet the in vitro transcription reaction directly onto the center of the slanted gel-bed surface (Figure 6). Do not allow the reaction mixture or the pipet tip to touch the sides of the column.

The sample should be pipetted slowly so that the drops are absorbed into the gel and do not flow down the sides of the gel bed. Avoid touching the gel-bed surface with the pipet tip. It is not necessary to replace the lid on the column.

12b. Centrifuge for 3 min at the calculated speed.

13b. Remove the spin column from the microcentrifuge tube.

The eluate contains the purified RNA.

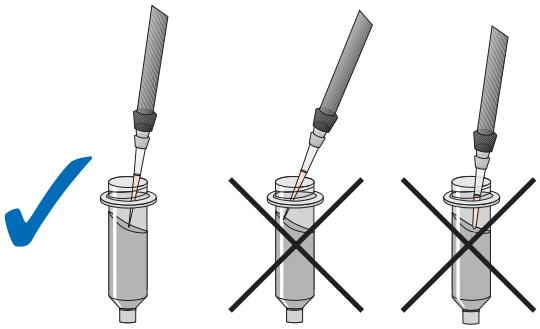


Figure 6 Instructions for sample application to the DyeEx 2.0 spin column.

14b. Thaw and store EasyXpress Insect Reaction Buffer, EasyXpress Insect Extract, EasyXpress Energy Mix, and RNase-Free Water on ice.

15b. Pipet together the components of the three translation reactions shown in Table 4 in three DNase- and RNase-free 1.5 ml microcentrifuge tubes. Label each tube clearly.

It is important to add the reaction components in the order shown in Table 4.

Table 4. Pipetting Scheme for High-Yield In Vitro Translation Reactions

Reagent	Target protein synthesis reaction	Positive control reaction	No template control reaction
RNase-Free Water	8 µl	8 µl	8 µl
EasyXpress Insect Reaction Buffer	5 µl	5 µl	5 µl
EasyXpress Insect Extract	20 µl	20 µl	20 µl
Template mRNA*	12 µl	12 µl	12 µl
Insect EasyXpress Energy Mix	5 µl	5 µl	5 µl
Total	50 µl	50 µl	50 µl

* From step 13b. Use an aliquot of the DyeEx spin column flow-through from the respective transcription reaction.

16b. Mix all components by vortexing and briefly centrifuge to collect the reactions at the bottom of the tubes.

17b. Incubate the in vitro translation reactions in a Thermomixer for 90 min at 27°C and 500 rpm.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocol in this handbook or molecular biology applications (see back page for contact information).

	Comments and suggestions
No target protein	
a) Poor quality or wrong quantity of DNA template	Check the concentration, integrity, and purity of the linearized DNA template. Prepare high-purity plasmid DNA with QIAGEN plasmid kits.
b) DNA template not optimally configured, or error in cloning	Check the sequence. Make sure that the start codon is in the right position for expression (see "Minimum template requirements", page 11). Ensure that the expression plasmid contains a T7 promoter.
c) In vitro transcription or in vitro translation is disrupted by expressed protein	Express control protein in the presence of the target protein. If expression of control protein is inhibited, it may not be possible to express the target protein using the EasyXpress Protein Synthesis System.
d) Rigid secondary structures in the mRNA inhibit initiation of translation	Include a 6xHis-tag coding sequence at the 5' end of the protein coding sequence. If the protein to be expressed already contains a tag, move the tag to the opposite terminus.
Low expression yield	
e) Poor quality or wrong quantity of DNA template	Check the concentration, integrity, and purity of the linearized DNA template. Prepare high-purity plasmid DNA with QIAGEN plasmid kits. Increase or reduce the amount of DNA in the in vitro transcription reaction by 0.5 µg per 25µl reaction. Alternatively titrate the volume of cleaned up mRNA added to the in vitro translation reaction between 6 and 20 µl, if using the high-yield protocol.

	Comments and suggestions
f) GC-rich mRNA	Denature the template mRNA prior to in vitro translation at 65°C for 3 min and immediately cool in an ice-water bath. This may increase the efficiency of translation, especially of GC-rich mRNA, by destroying local regions of secondary structure.

Appendix A: Purifying Affinity-Tagged Recombinant Proteins

Purifying 6xHis-tagged Proteins Using Ni-NTA Magnetic Agarose Beads

Recombinant proteins carrying a 6xHis-tag can be efficiently purified in a one-step affinity purification procedure using Ni-NTA Magnetic Agarose Beads. This protocol can be used for purifying 6xHis-tagged protein generated in a 50 µl EasyXpress Protein Synthesis Insect Kit reaction.

Materials and reagents to be supplied by the user

- EasyXpress in vitro protein synthesis reaction containing 6xHis-tagged target protein
- Ni-NTA Magnetic Agarose Beads (QIAGEN cat. no. 36111)
- Binding, wash, and elution buffers
- Microcentrifuge tubes for elution fractions
- Magnetic Separator (e.g., Single-Tube Magnet, QIAGEN cat. no. 36910)

Buffer compositions can be found in Appendix D on page 33.

Procedure

1. **Resuspend Ni-NTA Magnetic Agarose Beads by vortexing for 2 s and immediately pipet 50 µl 5% Ni-NTA Magnetic Agarose bead suspension into a 1.5ml reaction tube.**

   Note: care is necessary to ensure that constant amounts of beads are pipetted. The beads will settle if the suspension is not agitated regularly.
2. **Place the reaction tube on magnetic separator for 1 min. Carefully remove supernatant with a pipet.**
3. **Remove the tube from the magnetic separator and add 200 µl Ni-NTA Beads Binding Buffer. Briefly vortex, place the reaction tube on a magnetic separator for 1 min, and remove supernatant.**
4. **Pipet 50 µl in vitro translation reaction into the tube containing the magnetic beads.**
5. **Mix the suspension gently on an end-over-end shaker or shaker platform for 60 min at 4°C.**
6. **Place the tube on a magnetic separator for 1 min and remove supernatant with a pipet.**

   Tubes may be briefly centrifuged before placing on a magnetic separator, to collect droplets of suspension from the tube caps.

7. **Remove tube from the magnet, add 200 µl Ni-NTA Beads Wash Buffer, mix the suspension, place the tube on a magnetic separator for 1 min, and remove buffer.**
8. **Repeat step 7.**

   Buffer remaining after the final wash should be removed completely.
9. **Add 50 µl of Ni-NTA Beads Elution Buffer to the beads, mix the suspension, incubate the tube for 1 min on magnetic separator for 1 min, and collect eluate.**

   Tubes may be centrifuged before placing on the magnetic separator, to collect droplets of suspension from the tube caps.
10. **Repeat step 9.**

    Most of the 6xHis-tagged protein will elute in the first elution step.

Appendix B: Analyzing the Luciferase Positive-Control Translation Reaction

The positive-control reaction is performed using a DNA template that encodes luciferase. Luciferase is a monomeric protein with a molecular weight of 61 kDa, which is found in the cells of bioluminescent organisms and catalyzes the oxidation of luciferin and ATP, producing light. Only full-length luciferase is active.

For a luciferase activity assay using a commercially available kit (e.g., Promega Luciferase Assay Reagent, cat. no. E1483), use 10 µl crude luciferase translation reaction.

For western blot analysis, load 2 µl (chemiluminescent detection) or 8 µl (chromogenic detection) crude luciferase translation reaction onto a 12% SDS-PAGE gel, transfer the protein from the gel onto nitrocellulose, and detect the luciferase protein with an anti-luciferase antibody (e.g., Monoclonal Anti-Luciferase Antibody, Sigma, cat. no. L2164).

For quantification of expressed luciferase using incorporation of radioactively-labeled amino acids, see Appendix C.

Appendix C: Incorporating Radioactive Labels into Proteins for Quantification

Protein expressed using the EasyXpress Protein Synthesis Insect Kit can be quantified by incorporating radioactive amino acids (e.g., ^{14}C-leucine or ^{35}S-methionine). ^{14}C is more stable than ^{35}S and its use is recommended for accurate quantification. However, ^{35}S provides a stronger signal. It is recommended that 500 pmol ^{14}C-labeled leucine (^{14}C-Leu) is added per 50 µl reaction. A protocol and example calculation of protein yield is given below.

Materials and equipment to be supplied by user

- Linearized plasmid DNA encoding protein of interest
- Thermomixer
- 250 µM ^{14}C-labeled leucine (^{14}C-Leu, 100 cpm/pmol)

Procedure

1. **Perform a transcription reaction (see protocol on page 16).**
2. **Thaw EasyXpress Insect Reaction Buffer (blue screw-cap) on ice. Thaw RNase-free water (colorless screw-cap) and EasyXpress Positive-Control DNA (violet screw-cap) at room temperature (15–25°C).**
3. **For each reaction to be performed, thaw 20 µl of EasyXpress Insect Extract (colorless snap-cap) on ice.**

4. **Set up the three reactions detailed in the table below.**
   It is important to follow the order of addition given in the table.

Reagent	Target protein synthesis reaction	Positive control reaction	No template control reaction
RNase-free water	11 µl	11 µl	11 µl
EasyXpress Insect Reaction Buffer	5 µl	5 µl	5 µl
250 µM ^{14}C-Leu	4 µl	4 µl	4 µl
EasyXpress Insect Extract	20 µl	20 µl	20 µl
Template mRNA*	5 µl	5 µl	5 µl
EasyXpress Insect Energy Mix	5 µl	5 µl	5 µl
Total	**50 µl**	**50 µl**	**50 µl**

* From in vitro transcription step 5, page 17. Use an aliquot of the respective transcription reaction.

5. **Mix all components by vortexing and briefly centrifuge to collect the reactions at the bottom of the tubes.**
6. **Incubate the in vitro translation reactions in a Thermomixer for 90 min at 27°C and 500 rpm.**
7. **Use a 10 µl aliquot of each reaction for quantification of protein synthesis by TCA precipitation (see page 30).**
   Alternatively, the reactions can be separated by SDS-PAGE and analyzed by autoradiography after drying the gel.

Labeling with ^{35}S-Methionine

As an alternative to ^{14}C-leucine labeling, in vitro translated proteins can be labeled using ^{35}S-methionine (^{35}S-Met). A pipetting scheme for the reactions is given in the table below. Labeling proteins with ^{35}S-methionine gives stronger signals. We recommend using 1 µl of 15 µM ^{35}S-methionine (1000 mCi/mmol) for labeling each reaction.

Reagent	Target protein synthesis reaction	Positive control reaction	No template control reaction
RNase-free water	14 µl	14 µl	14 µl
EasyXpress Insect Reaction Buffer	5 µl	5 µl	5 µl
15 µM ^{35}S-Met	1 µl	1 µl	1 µl
EasyXpress Sf21 Insect Extract	20 µl	20 µl	20 µl
Template mRNA*	5 µl	5 µl	5 µl
EasyXpress Insect Energy Mix	5 µl	5 µl	5 µl
Total	**50 µl**	**50 µl**	**50 µl**

* From in vitro transcription step 5, page 17. Use an aliquot of the respective transcription reaction.

Determination of Protein Yield by TCA Precipitation and Scintillation Counting

This protocol can be used for accurate quantification of radioactively-labeled protein yields from EasyXpress Protein Synthesis Insect Kit reactions.

Materials and equipment to be supplied by the user

- 5% (w/v) trichloroacetic acid (TCA) and acetone
- Casein acid hydrolysate (e.g., Sigma, cat. no. A 2427)
- Glass microfibre filters (for example Whatman® GF/C)
- Vacuum manifold (e.g., Glass Microanalysis Filter Holder, Millipore cat. no. XX1002530 in combination with a vacuum pump)
- Scintillation cocktail (for example Ready Protein+™; Beckman Coulter, Inc., cat. no. 158727) and scintillation counter

Procedure

1. **Briefly vortex the in vitro translation reaction mixture and transfer a 10 µl aliquot to a 10 ml test tube.**
2. **Add 3 ml of 5% TCA solution containing 2% (w/v) casein acid hydrolysate.**
3. **Mix and incubate for 15 min at 90°C.**

   During this step radiolabeled aminoacyl-tRNA as well as peptidyl-tRNA will be hydrolyzed.
4. **Incubate on ice for at least 30 min to precipitate the synthesized proteins.**
5. **Collect the precipitate on a glass microfibre filter by using a vacuum manifold. Before starting wet the filter with a few drops of 5% (w/v) TCA.**
6. **Wash the filter 3 times with 2 ml aliquots of 5% (w/v) TCA.**
7. **Dry the filter by rinsing it 2 times with 3 ml aliquots of acetone.**
8. **Transfer the filter to a scintillation vial and add an appropriate volume of scintillation cocktail.**
9. **Shake the sample gently for 1 h at room temperature.**
10. **Count the sample in a liquid scintillation counter.**
11. **To determine the total radioactivity added to the reactions, vortex the protein synthesis reaction mixture, transfer a 10 µl aliquot onto a filter disc placed in a scintillation vial, add scintillation cocktail and count the sample in a liquid scintillation counter.**

    Note: to determine background protein synthesis, take aliquots from the no-template control reaction and treat them as described in steps 2–10.

Calculation of protein synthesis yield

$$\text{Percentage of } {}^{14}\text{C-Leu incorporated} = \frac{\text{counts TCA precipitation (cpm/}\mu\text{l) x 100}}{\text{counts unprecipitated sample (cpm/}\mu\text{l)}}$$

$$\text{Yield (}\mu\text{g/ml)} = \frac{\%{}^{14}\text{C -Leu incorporated x 0.01 x conc. Leu (}\mu\text{M) x mol. wt. protein (g/mol)}}{\text{Leu residues in protein x 1000}}$$

Example calculations

Template	Luciferase control DNA
Molecular weight	61,683 g/mol
Leu residues	51
Met residues	14

Labeling with ^{14}C-leucine

Leucine concentration	30 μM	unlabeled in the kit
^{14}C-Leu concentration	20 μM	
Total leucine concentration	50 μM	40 cpm/pmol

Measured radioactivity

TCA precipitated sample (10 μl)	2500 cpm = 250 cpm/μl
Total radioactivity (10 μl sample)	20,000 cpm = 2000 cpm/μl

$$\text{Percentage of } {}^{14}\text{C-Leu incorporated} = \frac{250 \times 100}{2000} = 12.5\%$$

$$\text{Yield (}\mu\text{g/ml)} = \frac{12.5\% \times 0.01 \times 50\,\mu\text{M} \times 61{,}683\ \text{g/mol}}{51 \times 1000} = 7.56\ \mu\text{g/ml}$$

Labeling with ^{35}S-methionine

Proteins labeled with ^{35}S-methionine gives stronger signals than ^{14}C-labeled proteins. A typical commercially available ^{35}S-methionine solution has a specific activity of 1 μCi/pmol = 2.22 x 10^6 cpm/pmol and a concentration of 15 μM. This example is based on the addition of 1 μl of ^{35}S-methionine solution (= 1 μCi) for a 50 μl in vitro translation reaction.

Methionine concentration	30 μM	unlabeled in the kit
^{35}S-Met concentration	0.3 μM	2.2 x 10^6 cpm/pmol
Total methionine concentration	30.3 μM	44,000 cpm/pmol

Measured radioactivity

TCA precipitated sample (10 μl)	33,000 cpm = 3300 cpm/μl
Total radioactivity (10 μl sample)	444,000 cpm = 44,000 cpm/μl

$$\text{Percentage of } ^{35}\text{S-Leu incorporated} = \frac{3300 \times 100}{44{,}000} = 7.5\%$$

$$\text{Yield } (\mu\text{g/ml}) = \frac{7.5\% \times 0.01 \times 30.3\ \mu\text{M} \times 61{,}683\ \text{g/mol}}{14 \times 1000} = 10.01\ \mu\text{g/ml}$$

Appendix D: Buffer Compositions

NPI-10-T (Ni-NTA Beads Binding Buffer, 1 liter):

50 mM NaH_2PO_4	6.90 g $NaH_2PO_4 \cdot H_2O$ (MW 137.99 g/mol)
300 mM NaCl	17.54 g NaCl (MW 58.44 g/mol)
10 mM imidazole	0.68 g imidazole (MW 68.08 g/mol)
0.05% (v/v) Tween® 20	5 ml of a 10% (v/v) Tween 20 stock solution

Adjust pH to 8.0 using NaOH.

NPI-20-T (Ni-NTA Beads Wash Buffer, 1 Liter):

50 mM NaH_2PO_4	6.90 g $NaH_2PO_4 \cdot H_2O$ (MW 137.99 g/mol)
300 mM NaCl	17.54 g NaCl (MW 58.44 g/mol)
20 mM imidazole	1.36 g imidazole (MW 68.08 g/mol)
0.05% (v/v) Tween 20	5 ml of a 10% (v/v) Tween 20 stock solution

Adjust pH to 8.0 using NaOH.

NPI-250-T (Ni-NTA Beads Elution Buffer, 1 Liter):

50 mM NaH_2PO_4	6.90 g $NaH_2PO_4 \cdot H_2O$ (MW 137.99 g/mol)
300 mM NaCl	17.54 g NaCl (MW 58.44 g/mol)
250 mM imidazole	17.0 g imidazole (MW 68.08 g/mol)
0.05% (v/v) Tween 20	5 ml of a 10% (v/v) Tween 20 stock solution

Adjust pH to 8.0 using NaOH.

Appendix E: Cloning Expression Sequences into the pIX4.0 Vector

The pIX4.0 cloning and expression vector has been specially developed to provide high expression rates in insect-cell lysates. It contains the T7 transcription promoter, optimized 5'- and 3'-untranslated regions (UTRs), a multiple cloning site (MCS), the T7 transcription terminator to stabilize mRNA against exonucleolytic digestion, several alternative restriction sites for plasmid linearization with blunt ends for effective run-off transcription, hybridization sequences for sequencing primers (M13 forward, reverse), an ampicillin resistance marker, and a high-copy origin of replication.

Cloning strategies

*Nco*I *Bse*RI *Xho*I *Not*I *Pst*I *Bgl*II *Spe*I

```
CCATGGGAGACCCCTCCTCGAGCGGCCGCCTGCAGATCTAAATAATAAGTAATTAACTAGT
GGTACCCTCTGGGGAGGAGCTCGCCGGCGGACGTCTAGATTTATTATTCATTAATTGATCA
   Met Gly Asp Pro Ser Ser Ser Gly Arg Leu Gln Ile
```

The pIX4.0 multiple cloning site

If the *Nco*I cloning site is used, it should be noted that because the G 3' of the ATG start codon is an absolute requirement for *Nco*I restriction, sequences that are cloned into the pIX4.0 vector using the *Nco*I restriction site will start with an N-terminus Met followed by the amino acid Gly, Glu, Asp, Ala, or Val. If the *Xho*I, *Not*I, *Pst*I, *Bgl*II, or *Spe*I cloning sites are used, it should be noted that because translation commences at the ATG start codon, additional amino acids will be added to the N-terminus of the native protein sequence. If native structure proteins, which are free of vector-encoded amino acids, are required, the *Bse*RI cloning site should be used.

The cloning options can be summarized as follows:

- Protein sequence starts with an N-Terminus Met followed by the amino acid Gly, Glu, Asp, Ala, or Val. See "Cloning into pIX4.0 via *Nco*I", page 35.
- Proteins are synthesized free of any additional vector amino acids. See "Cloning into pIX4.0 via *Bse*RI", page 38.
- Protein contains up to 6 vector-encoded N-terminal amino acids. See "Cloning into pIX4.0 via *Xho*I, *Not*I, *Pst*I, *Bgl*II", page 41.
- Protein is synthesized with an N-terminal 6xHis or *Strep*-tag®. See "PCR-mediated addition of affinity-tag sequences", page 44.
- Protein is synthesized with a C-terminal 6xHis or *Strep*-tag. See "PCR-mediated addition of affinity-tag sequences", page 44.

Cloning into pIX4.0 via *Nco*I

For the PCR, a sense primer with the following structure should be synthesized:

5' - XXXXXX<u>CC**ATG**G</u>(M_{20})–3'
*Nco*I

Sense primer

The restriction enzyme recognition sequence is underlined and the translation start codon ATG is in bold. X can be any base; the presence of this short sequence increases digestion efficiency. (M_{20}) corresponds to bases 5–24 of the target protein coding sequence.

For the PCR, an antisense primer with the following structure should be synthesized:

5' - XXXXXX<u>AGATCT</u>**TTA**(N_{20})3'
*Bgl*II Stop

Antisense primer

The restriction enzyme recognition sequence is underlined and the translation stop is in bold. X can be any base; the presence of this short sequence increases digestion efficiency. (N_{20}) corresponds to the antisense sequence of the last 20 bases of the target protein coding sequence.

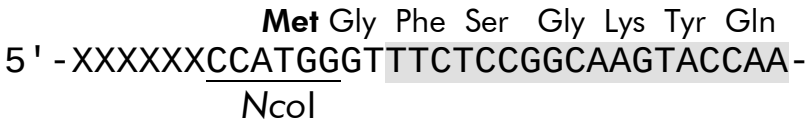
Example: Primer design for cloning of FABP using the *Nco*I and *Bgl*II cloning sites

The beginning and ends of the protein coding sequence for fatty acid binding protein (FABP) are shown below. The bases that will serve as PCR primer binding sites (bases 5–24 [sense] and the last 20 bases [antisense]) are in bold.

Met Ser Phe Ser Gly Lys Tyr Gln Lys Arg Ile Ser Lys Arg Ile Stop
5'–ATGA**GTTTCTCCGGCAAGTACCAA**C...AAGAGAATCAGCAAGAGAATTTGA–3'
3'–TACTCAAAGAGGCCGTTCATGGTTG...T**TCTCTTAGTCGTTCTCTTAA**ACT–5'

Coding sequence of FABP.

For the PCR the following sense primer is constructed. Native target protein sequence is shaded.



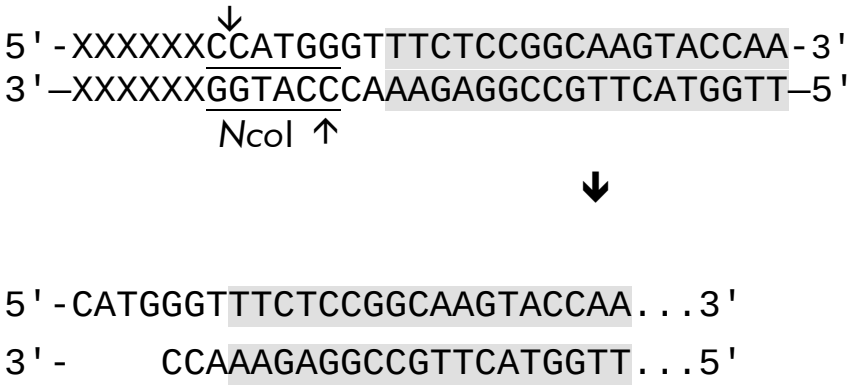
Sense primer

It should be noted that due to the presence of the G 3' of the ATG start codon, the Ser residue in the native sequence will be converted to a Gly.

For the PCR the following antisense primer is constructed. Native target protein sequence is shaded.

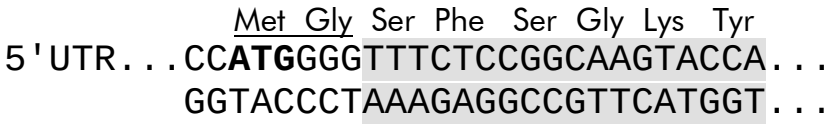
Antisense primer

As a first stage of the cloning procedure, the PCR product is digested with *Nco*I. Native target protein sequence is shaded.



The vector is then digested with *Nco*I.

The digested PCR product and vector are then ligated. Native target protein sequence is shaded. Vector-encoded amino acids are underlined.



Cloning into pIX4.0 via *Bse*RI

For the PCR, a sense primer with the following structure should be synthesized:

5'-XXXXXXGAGGAGGTCTCCC**ATG**(M_{20})–3'

*Bse*RI Met

Sense primer

The restriction enzyme recognition sequence is underlined and the translation start codon ATG is in bold. X can be any base; the presence of this short sequence increases digestion efficiency. (M_{20}) corresponds to bases 4–23 of the target protein coding sequence.

For the PCR, an antisense primer with the following structure should be synthesized:

5'-XXXXXXAGATCT**TTA**(N_{20})–3'

*Bgl*II Stop

Antisense primer

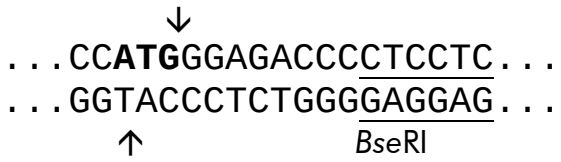
The restriction enzyme recognition sequence is underlined and the translation stop is in bold. X can be any base; the presence of this short sequence increases digestion efficiency. (N_{20}) corresponds to the antisense sequence of the last 20 bases of the target protein coding sequence.

A section of the resulting double-stranded PCR product is shown below. It should be noted that *Bse*RI cuts at a staggered site 10 and 8 bases away from its recognition site (arrowed). The recognition site is underlined and the ATG start codon appears in bold.

```
                                    ↓
5' XXXXXXGAGGAGGTCTCCC**ATG**NNN...3'
   XXXXXXCTCCTCCAGAGGGTACNNN...5'
          BseRI          ↑
```

PCR product sequence

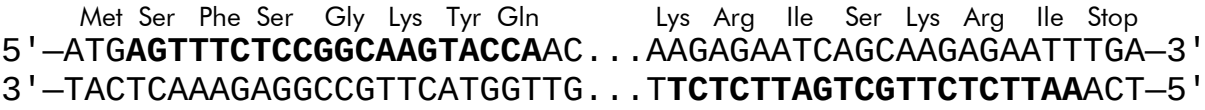
A section of the pIX4.0 vector MCS is shown below. As before, *Bse*RI cuts at a staggered site 10 and 8 bases away from its recognition site (arrowed). In this case the recognition site is located downstream of the ATG start codon (bold). The recognition site is underlined.



pIX4.0 vector sequence.

Example: Primer design for cloning of FABP using the *Bse*RI and *Bgl*II cloning sites

The beginning and ends of the protein coding sequence for fatty acid binding protein (FABP) are shown below. The bases that will serve as PCR primer binding sites (bases 4–23 [sense] and the last 20 bases [antisense]) are in bold.



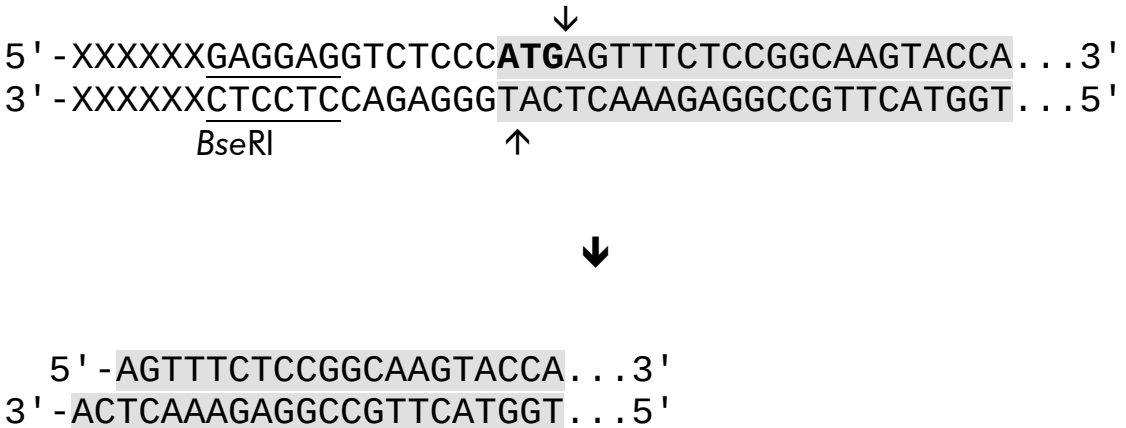
Coding sequence of FABP.

For the PCR the following primers are constructed. Native target protein sequence is shaded.

Sense primer

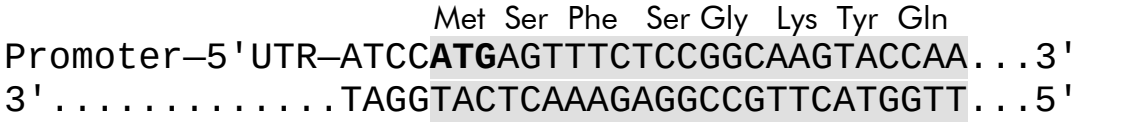
Antisense primer

As a first stage of the cloning procedure, the PCR product is digested with *Bse*RI. Native target protein sequence is shaded.



The vector is then digested with *Bse*RI.

The digested PCR product and vector are then ligated. Native target protein sequence is shaded.



Cloning into pIX4.0 via *Xho*I, *Not*I, *Pst*I, or *Bgl*II

Sequences that are cloned into the pIX4.0 vector using the *XhoI, NotI, PstI, or BglII* restriction sites will contain up to six vector encoded amino acids (see below). Suggested PCR primers for cloning are given below. Restriction sites are underlined and the translation start codon is in bold. X can be any base, the presence of this short sequence increases digestion efficiency. (M_{20}) corresponds to bases 4–23 of the target protein coding sequence. (N_{20}) corresponds to the final 20 coding bases of the target protein coding sequence.

Cloning into pIX4.0 via *Xho*I

For the PCR, a sense primer with the following structure should be synthesized:

5'-XXXXXX<u>CTCGAG</u>C(M_{20})-3'
*Xho*I

Sense primer

The restriction enzyme recognition sequence is underlined. X can be any base; the presence of this short sequence increases digestion efficiency. (M_{20}) corresponds to bases 4–23 of the target protein coding sequence. The base C between the *Xho*I recognition sequence and the protein coding sequence is required to ensure that the coding sequence codons are shifted into frame.

Example: Primer design for cloning of FABP using the *Xho*I and *Bgl*II cloning sites

The beginning and ends of the protein coding sequence for fatty acid binding protein (FABP) are shown below. The bases that will serve as PCR primer binding sites (bases 4–23 [sense] and the last 20 bases [antisense]) are in bold.

Met Ser Phe Ser Gly Lys Tyr Gln Lys Arg Ile Ser Lys Arg Ile Stop
5'–ATG**AGTTTCTCCGGCAAGTACCA**AC...AAGAGAATCAGCAAGAGAATTTGA–3'
3'–TACTCAAAGAGGCCGTTCATGGTTG...T**TCTCTTAGTCGTTCTCTTAA**ACT–5'

Coding sequence of FABP.

For the PCR the following primers are constructed. Native target protein sequence is shaded.

```
                  Ser  Ser  Ser  Phe  Ser  Gly  Lys  Tyr
5'-XXXXXXCTCGAGCAGTTTCTCCGGCAAGTACCA-3'
          XhoI
```

Sense primer

```
                              Stop  Ile  Arg  Lys  Ser  Ile  Arg
5'-XXXXXXAGATCTTTAAATTCTCTTGCTGATTCTCT-3'
          BglII
```

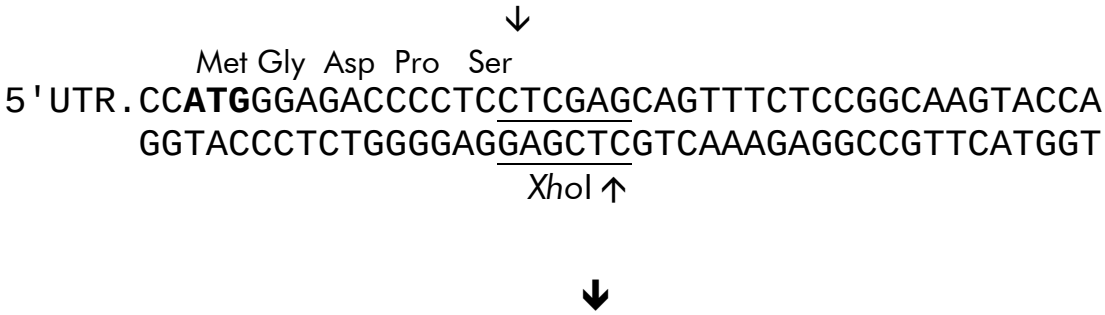
Antisense primer

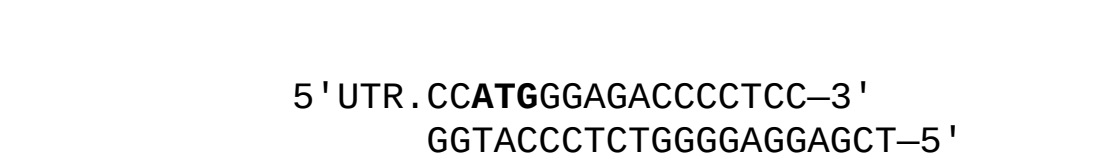
As a first stage of the cloning procedure, the PCR product is digested with *Xho*I. Native target protein sequence is shaded.

```
          ↓
5'-XXXXXXCTCGAGCAGTTTCTCCGGCAAGTACCA...3
3'-XXXXXXGAGCTCGTCAAAGAGGCCGTTCATGGT...5'
          XhoI  ↑
```

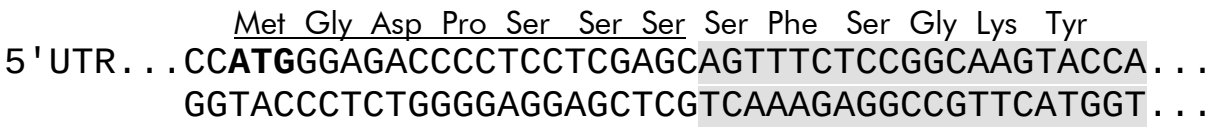
```
5'-TCGAGCAGTTTCTCCGGCAAGTACCA...3'
3'-    CGTCAAAGAGGCCGTTCATGGT...5'
```

The vector is then digested with *Xho*I.





The digested PCR product and vector are then ligated. Native target protein sequence is shaded. Vector-encoded amino acids are underlined.



PCR-mediated addition of affinity-tag sequences

The PCR primers listed below can be used to add affinity-tag coding sequences to expression constructs. Start codons are in bold, and restriction enzyme recognition sites are underlined. If the target protein coding bases lead to the formation of mRNA secondary structures, altering the amino acid codons may improve results.

Sense primers for addition of N-terminal affinity tags

Using the NcoI cloning site

N-terminal *Strep*-tag

```
                Met Asp Trp  Ser His Pro Gln  Phe Glu Lys Ser  Ala
5'-XXXXXXCCATGGATTGGTCTCATCCGCAATTCGAAAAAAGCGCT(N20)-3'
```

N-terminal 6xHis tag

```
                Met Asp His  His His His His  His
5'-XXXXXXCCATGGATCATCATCACCATCACCAC(N20)-3'
```

Using the BseRI cloning site

N-terminal *Strep*-tag

```
                          Met Trp  Ser His Pro Gln  Phe Glu Lys Ser  Ala
XXXXXXGAGGAGGTCTCCCATGTGGTCTCATCCGCAATTCGAAAAAAGCGCT(N20)
```

N-terminal 6xHis tag

```
                               Met His  His His His His  His
5' XXXXXXGAGGAGGTCTCCCATGCATCATCACCATCACCAC(N20)-3'
```

Using the XhoI cloning site

N-terminal *Strep*-tag

```
               Ser  Ser Trp  Ser His  Pro Gln Phe  Glu Lys  Ser Ala
5'-XXXXXXCTCGAGCTGGTCTCATCCGCAATTCGAAAAAAGCGCT(N20)-3'
```

N-terminal 6xHis tag

```
               Ser  Ser His  His His  His  His His
5'-XXXXXXCTCGAGCCATCATCACCATCACCAC(N20)-3'
```

Antisense primers for addition of C-terminal affinity tags

Using the *Bgl*II cloning site

C-terminal *Strep*-tag

Stop Lys Glu Phe Gln Pro His Ser Trp Ala Ser

5'-XXXXXXAGATCT**TTA**TTTTTCGAATTGCGGATGAGACCAAGCGCT(N_{20})-3'

C-terminal 6xHis tag

Stop His His His His His His

5'-XXXXXXAGATCT**TTA**GTGGTGATGGTGATGATG(N_{20})-3'

Additional cloning options for pIX4.0

If the recognition sequence for *Nco*I is present in the target protein's coding sequence, or codon for the N-terminal amino acid cannot commence with a guanine, *Nco*I cannot be used for cloning. In such cases, the recognition sequence for *Bsp*HI (TCATGA) or *Pci*I (ACATGT) can be added to the target gene sequence by PCR; of course these sequences should not appear in the target protein sequence. The *Bsp*HI or *Pci*I restricted insert can be ligated into the *Nco*I restricted vector. Using *Bsp*HI or *Pci*I for restriction dictates that the first base of the N-terminal codon is A or T respectively.

If the recognition sequence for *Bse*RI is present in the target protein's coding sequence, *Bse*RI cannot be used for cloning. In such cases, the recognition sequence for *Bts*I or *Bsr*DI (see below) can be added to the target gene sequence by PCR; of course these sequences should not appear in the target protein sequence.

*Bts*I	GCAGTGNN CGTCACNN	GCAGTG**TG** CGTCACAC
*Bsr*DI	GCAATGNN CGTTACNN	GCAATG**TG** CGTTACAC

The *Bts*I or *Bsr*DI restricted insert can be ligated into the *Bse*RI restricted vector if the DNA sense strand contains TG at the position indicated above in bold. The TG motif forms the second and third position of the ATG start codon. Additional restriction enzymes that can be used for restriction of PCR products that can be ligated into a *Bse*RI restricted vector are *Bce*AI, *Bpm*I, *Bpu*EI, *Bsg*I, *Eci*I, or *Mme*I.

References

1) Kubick, S., Merk, H., Stiege, W., von Groll, U., Drees, J., and Schaefer, F.* Insect-cell based in vitro synthesis of posttranslationally modified proteins. QIAGEN News 2004. 41.

2) Sambrook, J., Fritsch, E., and Maniatis, T. (1989) *Molecular Cloning — A laboratory Manual*. 2nd Ed. Cold Spring Harbor. Cold Spring Harbor Laboratory Press.

Ordering Information

Product	Contents	Cat. no.
EasyXpress Protein Synthesis Insect Kit	For 20 x 50 µl reactions: *Spodoptera frugiperda* insect cell extract, in vitro transcription reaction components, reaction buffers, RNase-free water, and positive-control DNA	32552
EasyXpress pIX4.0 Vector	25 µg vector DNA for efficient synthesis of proteins using the EasyXpress Protein Synthesis Insect Kit	32713
Related products		
EasyXpress Protein Synthesis Mega Kit	For 2 x 5 ml reactions: *E. coli* extract, reaction buffers, amino acid mix w/o methionine, methionine, RNase-free water, gel-filtration columns, and reaction flasks	32516
EasyXpress Protein Synthesis Mini Kit	For 20 x 50 µl reactions: *E. coli* extract, reaction buffer, RNase-free Water, and positive-control DNA	32502
EasyXpress Protein Synthesis Maxi Kit	For reactions up to 4000 µl: 4 x 350 µl *E. coli* extract, reaction buffer, RNase-free Water, and positive-control DNA	32506
EasyXpress Linear Template Kit (20)	For 20 two-step PCRs: ProofStart DNA Polymerase, buffer, RNase-free Water, Q-solution, XE-Solution, positive-control DNA, and PCR primers	32703
EasyXpress Site-Specific Biotin Kit	For 5 x 25 µl reactions: *E. coli* extract, reaction buffer, RNase-free Water, biotinyl-lysyl tRNA (amber), and positive-control DNA	32602
EasyXpress Random Biotin Kit	For 20 x 50 µl reactions: *E. coli* extract, reaction buffers, RNase-free Water, biotinyl-lysyl tRNA (Phe), and positive-control DNA	32612

Product	Contents	Cat. no.
EasyXpress NMR Protein Synthesis Kit	For 2 x 5 ml reactions: *E. coli* extract, reaction buffers, amino acid mix w/o Arg, Lys, Ser, Thr, Val (supplied as individual amino acids), RNase-free water, gel-filtration columns, and reaction flasks	32526
EasyXpress NMR Protein Synthesis Kit – A	For 2 x 5 ml reactions: *E. coli* extract, reaction buffers, amino acid mix w/o Ala (to be supplied by user), RNase-free water, gel-filtration columns, and reaction flasks	32530
EasyXpress NMR Protein Synthesis Kit – N	For 2 x 5 ml reactions: *E. coli* extract, reaction buffers, amino acid mix w/o Asn (to be supplied by user), RNase-free water, gel-filtration columns, and reaction flasks	32531
EasyXpress NMR Protein Synthesis Kit – D	For 2 x 5 ml reactions: *E. coli* extract, reaction buffers, amino acid mix w/o Asp (to be supplied by user), RNase-free water, gel-filtration columns, and reaction flasks	32532
EasyXpress NMR Protein Synthesis Kit – C	For 2 x 5 ml reactions: *E. coli* extract, reaction buffers, amino acid mix w/o Cys (to be supplied by user), RNase-free water, gel-filtration columns, and reaction flasks	32533
EasyXpress NMR Protein Synthesis Kit – E	For 2 x 5 ml reactions: *E. coli* extract, reaction buffers, amino acid mix w/o Glu (to be supplied by user), RNase-free water, gel-filtration columns, and reaction flasks	32534
EasyXpress NMR Protein Synthesis Kit – Q	For 2 x 5 ml reactions: *E. coli* extract, reaction buffers, amino acid mix w/o Gln (to be supplied by user), RNase-free water, gel-filtration columns, and reaction flasks	32535
EasyXpress NMR Protein Synthesis Kit – G	For 2 x 5 ml reactions: *E. coli* extract, reaction buffers, amino acid mix w/o Gly (to be supplied by user), RNase-free water, gel-filtration columns, and reaction flasks	32536
EasyXpress NMR Protein Synthesis Kit – H	For 2 x 5 ml reactions: *E. coli* extract, reaction buffers, amino acid mix w/o His (to be supplied by user), RNase-free water, gel-filtration columns, and reaction flasks	32537

Product	Contents	Cat. no.
EasyXpress NMR Protein Synthesis Kit – I	For 2 x 5 ml reactions: *E. coli* extract, reaction buffers, amino acid mix w/o Ile (to be supplied by user), RNase-free water, gel-filtration columns, and reaction flasks	32538
EasyXpress NMR Protein Synthesis Kit – L	For 2 x 5 ml reactions: *E. coli* extract, reaction buffers, amino acid mix w/o Leu (to be supplied by user), RNase-free water, gel-filtration columns, and reaction flasks	32539
EasyXpress NMR Protein Synthesis Kit – M	For 2 x 5 ml reactions: *E. coli* extract, reaction buffers, amino acid mix w/o Met (to be supplied by user), RNase-free water, gel-filtration columns, and reaction flasks	32540
EasyXpress NMR Protein Synthesis Kit – F	For 2 x 5 ml reactions: *E. coli* extract, reaction buffers, amino acid mix w/o Phe (to be supplied by user), RNase-free water, gel-filtration columns, and reaction flasks	32541
EasyXpress NMR Protein Synthesis Kit – P	For 2 x 5 ml reactions: *E. coli* extract, reaction buffers, amino acid mix w/o Pro (to be supplied by user), RNase-free water, gel-filtration columns, and reaction flasks	32542
EasyXpress NMR Protein Synthesis Kit – W	For 2 x 5 ml reactions: *E. coli* extract, reaction buffers, amino acid mix w/o Trp (to be supplied by user), RNase-free water, gel-filtration columns, and reaction flasks	32543
EasyXpress NMR Protein Synthesis Kit – Y	For 2 x 5 ml reactions: *E. coli* extract, reaction buffers, amino acid mix w/o Tyr (to be supplied by user), RNase-free water, gel-filtration columns, and reaction flasks	32544

Product	Contents	Cat. no.
Protein purification		
Ni-NTA Superflow Columns (12 x 1.5 ml)*	For 12 6xHis-tagged protein preps: 12 polypropylene columns containing 1.5 ml Ni-NTA Superflow	30622
Ni-NTA Superflow (25 ml)*	25 ml nickel-charged resin (max. pressure: 140 psi)	30410
Ni-NTA Agarose (25 ml)*	25 ml nickel-charged resin (max. pressure: 2.8 psi)	30210
Strep-Tactin Superflow (2 ml)*	For batch and HPLC purification of *Strep*-tagged proteins: 2 ml *Strep*-Tactin-charged Superflow (max. pressure: 140 psi)	30001
Polypropylene Columns (5 ml)	50/pack, 5 ml capacity	34964
QIArack	1 rack for holding gel-filtration columns or affinity-resin filled polypropylene columns	19015
Ni-NTA Magnetic Agarose Beads (2 x 1 ml)*	2 x 1 ml nickel-charged magnetic agarose beads (5% suspension)	36111
Strep-Tactin Magnetic Beads (2 x 1 ml)*	For micro-scale purification of *Strep*-tagged proteins: 2 x 1 ml *Strep*-Tactin-charged magnetic agarose beads (10% suspension)	36311
Protein detection		
Penta-His HRP Conjugate Kit	125 µl Penta-His HRP Conjugate, 5 g Blocking Reagent, 50 ml Blocking Reagent Buffer (10x concentrate)	34460
Strep-tag Antibody (100 ug)	Mouse monoclonal antibody that recognizes the *Strep*-tag II epitope; lyophilized, for 1000 ml working solution	34850
6xHis Protein Ladder	6xHis-tagged marker proteins (lyophilized, for 50-100 lanes on western blots)	34705

* Larger sizes available; please inquire

Product	Contents	Cat. no.
Plasmid DNA purification		
HiSpeed Plasmid Midi Kit (25)*	25 HiSpeed Midi Tips, 25 QIAfilter Midi Cartridges, 25 QIAprecipitator Midi Modules plus Syringes, Reagents, Buffers	12643
QIAfilter Plasmid Midi Kit (25)*	25 QIAGEN-tip 100, Reagents, Buffers, 25 QIAfilter Midi Cartridges	12243
QIAGEN Plasmid Midi Kit (25)*	25 QIAGEN-tip 100, Reagents, Buffers	12143
Linearized plasmid DNA purification		
QIAquick Nucleotide Removal Kit (50)*	50 QIAquick Spin Columns, Buffers, Collection Tubes (2 ml)	28304

* Larger sizes available; please inquire

Notes

Notes

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