

July 2005

EasyXpress™ Large-Scale Synthesis Handbook

EasyXpress Protein Synthesis Mega Kit

EasyXpress NMR Protein Synthesis Kits

For large-scale production of proteins by
in vitro translation for structural analysis



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Strep-tag™ and *Strep-Tactin*™ (IBA GmbH)

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Strep-tag technology for protein purification and detection is covered by US patent 5,506,121, UK patent 2272698 and French patent 93 13 066; *Strep-Tactin* is covered by US patent 6,103,493.

Procedures for production of a protein containing seleno-methionine suitable for structure determination by crystallography and X-ray analysis may require a license under United States patent application US 2002/168705 or a subsequently granted patent or corresponding intellectual property.

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Kit Contents

EasyXpress NMR Protein Synthesis Kit		Cat. no. 32526
For 2 x 5 ml reactions		
Box 1 of 3		
<i>E. coli</i> Extract (NMR) (yellow screw-cap)		2 x 1.75 ml
Reaction Buffer without amino acids (12 ml reaction flask)		2 x 1.9 ml
Feeding Solution (NMR) (violet screw cap)		2 x 0.9 ml
Energy Mix (red screw cap)		2 x 1.1 ml
RNase-free Water (colorless screw-cap)		1 x 1.9 ml
Equilibration/Elution Buffer		2 x 59 ml
Box 2 of 3		
Gel Filtration Columns, Bed Volume 17 ml		2 columns
Reaction Flasks		2 flasks
Handbook		1
Box 3 of 3		
Amino Acid Mix (NMR) (orange screw-cap)		2 x 1.275 ml
Threonine (240 mM) (green screw-cap)		2 x 85 μ l
Lysine (240 mM) (green screw-cap)		2 x 85 μ l
Arginine (240 mM) (green screw-cap)		2 x 85 μ l
Valine (240 mM) (green screw-cap)		2 x 85 μ l
Serine (240 mM) (green screw-cap)		2 x 85 μ l

EasyXpress NMR Protein Synthesis Kit – indicated amino acid For 2 x 5 ml reactions	Cat. no.
EasyXpress NMR Protein Synthesis Kit – A (Ala)	32530
EasyXpress NMR Protein Synthesis Kit – N (Asn)	32531
EasyXpress NMR Protein Synthesis Kit – D (Asp)	32532
EasyXpress NMR Protein Synthesis Kit – C (Cys)	32533
EasyXpress NMR Protein Synthesis Kit – E (Glu)	32534
EasyXpress NMR Protein Synthesis Kit – Q (Gln)	32535
EasyXpress NMR Protein Synthesis Kit – G (Gly)	32536
EasyXpress NMR Protein Synthesis Kit – H (His)	32537
EasyXpress NMR Protein Synthesis Kit – I (Ile)	32538
EasyXpress NMR Protein Synthesis Kit – L (Leu)	32539
EasyXpress NMR Protein Synthesis Kit – M (Met)	32540
EasyXpress NMR Protein Synthesis Kit – F (Phe)	32541
EasyXpress NMR Protein Synthesis Kit – P (Pro)	32542
EasyXpress NMR Protein Synthesis Kit – W (Trp)	32543
EasyXpress NMR Protein Synthesis Kit – Y (Tyr)	32544
Box 1 of 3 — as Cat. no. 32526	
Box 2 of 3 — as Cat. no. 32526	
Box 3 of 3	
Amino Acid Mix (without indicated amino acid) (orange screw-cap)	2 x 1.275 ml

EasyXpress Protein Synthesis Mega Kit
For 2 x 5 ml reactions

Cat. no. 32516

Box 1 of 2

<i>E. coli</i> Extract (Mega) (white screw-cap)	2 x 1.75 ml
Reaction Buffer without methionine (12 ml reaction flask)	2 x 1.9 ml
Methionine (60 mM) (green screw-cap)	2 x 0.3 ml
Feeding Solution (Mega) (blue screw cap)	2 x 1.7 ml
Energy Mix (red screw cap)	2 x 1.1 ml
RNase-free Water (colorless screw-cap)	1 x 1.9 ml
Equilibration/Elution Buffer	2 x 59 ml

Box 2 of 2

Gel Filtration Columns, Bed Volume 17 ml	2 columns
Reaction Flasks (50 ml)	2 flasks
Handbook	1

Storage and Stability

The EasyXpress Protein Synthesis Mega Kit Box 1 and the EasyXpress NMR Protein Synthesis Kit Boxes 1 and 3 are shipped on dry ice.

The EasyXpress Mega and NMR Protein Synthesis Box 2 is shipped at ambient temperature. Upon arrival, store the boxes according to the table below.

Box number	Storage temperature
1	-70°C to -80°C
2	2-8°C
3	-20°C or -70°C to -80°C

When stored under the above conditions and handled correctly, both kits can be stored for at least 6 months without showing any reduction in performance.

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 that can be used for a wide variety of downstream applications, including activity assays, protein–protein interaction studies, the expression and analysis of open reading frames, and structural and mutational analysis. Larger scale in vitro translation reactions provide unique possibilities for rapid production of large amounts of recombinant proteins suitable for a range of downstream applications, including structural analysis. To address these issues, QIAGEN has developed EasyXpress products for large scale production of up to 5 mg of recombinant protein.

The effective incorporation of stable-isotope–labeled amino acids into in vitro synthesized proteins allows structural determination in solution by high-resolution NMR spectroscopy (1–3). The EasyXpress NMR Protein Synthesis Kit allows incorporation of the stable-isotope–labeled amino acids threonine, arginine, valine, serine, or lysine. Labeling of these amino acids minimizes isotope scrambling effects (distribution of isotopic label to other amino acids due to amino acid metabolism). Fifteen further EasyXpress NMR Kits are available for incorporation of any of the remaining 15 amino acids with an isotopic label. It should be noted that although the EasyXpress *E. coli* Extract production procedure drastically reduces amino acid metabolism under in vitro expression conditions (compared with the in vivo situation), a residual metabolizing activity may be observed in isolated cases, which may lead to a certain degree of isotope scrambling.

The EasyXpress Protein Synthesis Mega Kit allows rapid production of milligram quantities of recombinant protein for a wide range of applications, including comprehensive functional studies, aptamer selection studies, and animal immunization. In addition, the kit allows incorporation of the unnatural amino acid selenomethionine. The incorporation of the unnatural, cytotoxic amino acid selenomethionine into in vitro synthesized proteins facilitates phasing of crystal protein structures and atomic structure determination using X-ray crystallography (3, 4).

The EasyXpress System

EasyXpress Mega and NMR Kits use highly productive *E. coli* lysates, which contain all translational machinery components (i.e., ribosomes, ribosomal factors, tRNAs, aminoacyl-tRNA synthetases, etc.) as well as T7 RNA polymerase. It is a coupled transcription–translation system that can be used to express full-length proteins from T7 or *E. coli* promoters in a batch reaction using supercoiled DNA templates.

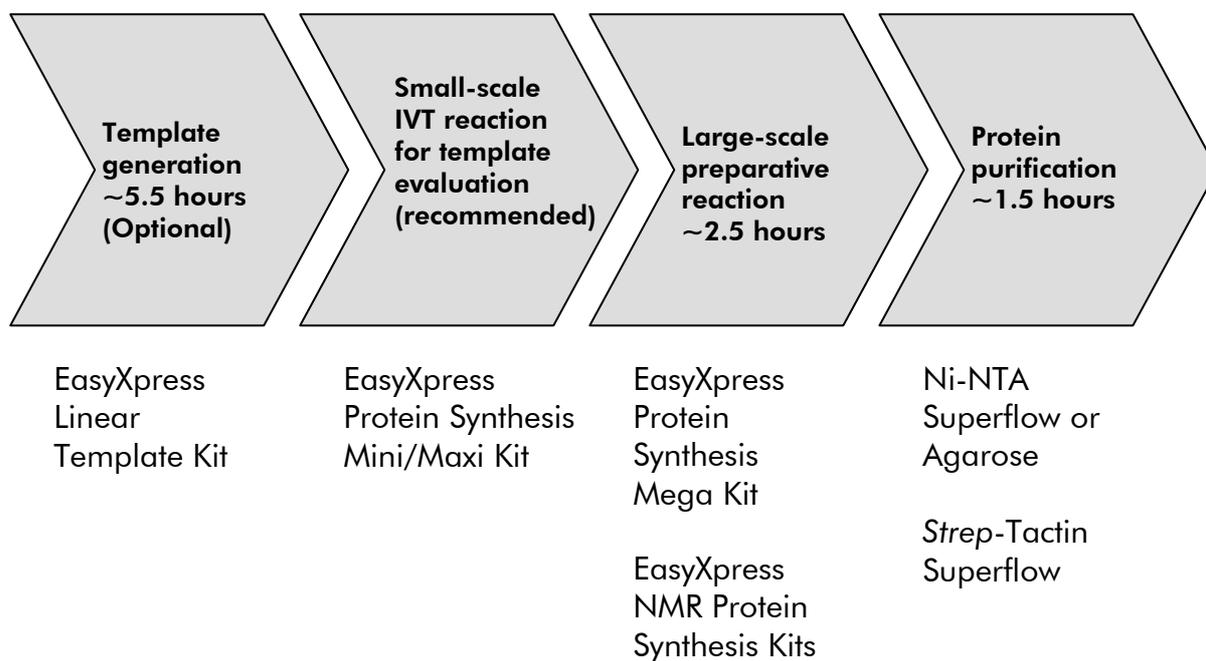
Large-Scale Protein Synthesis for Structural Proteomics Projects

To generate preparative yields of protein, the high-molecular-weight translation components in the reaction are recycled after a 1-hour batch synthesis and used in a second 1-hour batch synthesis (see flowchart, page 14). The recycling procedure is performed using a gel-filtration column. During the recycling step, low-molecular-weight reaction components (e.g., inorganic phosphate) that inhibit the *in vitro* translation reaction are removed from the high-molecular-weight translation components. Before the second *in vitro* translation reaction, the recycled high-molecular-weight translation components are supplemented with missing components, such as energy providers and amino acids. The short (in contrast to other [semi-]continuous systems) reaction time of 2 hours delivers high-quality proteins and reduces detrimental effects, such as degradation or precipitation. In addition, the short reaction time minimizes isotope exchange and breakdown or metabolism of isotopically labeled amino acids.

The EasyXpress Mega and NMR Protein Synthesis Kits yield up to 5 mg soluble recombinant protein from a reaction volume of 2 x 5 ml. Before using the EasyXpress Mega or NMR Protein Synthesis Kit, we recommend evaluating a plasmid expression template's suitability to produce soluble protein in high yields in a small-scale test reaction using the EasyXpress Protein Synthesis Mini or Maxi Kit. The relative protein yield (mg protein/ml reaction) typically increases by a factor of 2 in a large-scale reaction.

Although the EasyXpress system has been developed to give the highest yields of active and soluble protein, it may be possible to further optimize the synthesis procedure for individual proteins, e.g., the total expression or the solubility may be increased by including additives in the synthesis reaction (see EasyXpress Protein Synthesis Workflow, page 11, and Appendix A, page 38).

Workflow for Large-Scale Synthesis of Recombinant Protein Using the EasyXpress NMR or Mega Kit



DNA Templates

EasyXpress kits can be used to express proteins from a variety of DNA templates, as long as they contain a T7 or other strong *E. coli* promoter (e.g., T5) upstream from the coding sequence and a ribosome binding site. We recommend using supercoiled DNA plasmids as expression vectors. Vectors such as pET3d (Novagen) are a suitable basis for generating expression constructs.

Plasmid DNA

T7 promoter-based constructs, including the pET plasmid series (Novagen), are a suitable basis for generating expression constructs. Such vectors provide mRNA-stabilizing secondary structures in the 5' and 3' untranslated regions that play an important role in increasing the efficiency of expression.

Suitable vectors are not restricted to T7 promoter-based constructs. QIAGEN's pQE vectors have also been used successfully with EasyXpress *E. coli*-based kits.

Before use in a large-scale reaction, each expression vector should be tested in small-scale reactions (see page 38) to ensure efficient protein synthesis. The following table gives an overview of which vectors have successfully been used with EasyXpress kits.

Vector	Promoter	Protein(s) successfully synthesized
pET3d	T7	Several different proteins
pET15b	T7	Cytohesin-1/SEC7*
pET43	T7	NusA
pIX2.0 [†]	T7	EF-Ts
TAGZyme pQE-2	T5	TNF α
pQE-30	T5	Thioredoxin
pIVEX series	T7	GFP

* Expression construct kindly provided by Michael Blind, NascaCell IP GmbH, Munich, Germany.

[†] Available from QIAGEN Technical Service departments upon request. This vector is also suitable for cloning PCR templates generated using the EasyXpress Linear Template Kit.

Purification of plasmid DNA expression templates

Greatest yields of protein are obtained using template DNA of the highest purity. High-purity plasmid DNA can easily be obtained with the QIAGEN HiSpeed[®], QIAfilter, and QIAGEN Plasmid Kits. DNA prepared using the standard alkaline lysis method described by Sambrook, Fritsch and Maniatis (5) may be sufficiently pure, but DNA must be free of RNases.

For EasyXpress Mega and NMR protein synthesis reactions, the concentration of plasmid DNA in each 5 ml in vitro translation reaction should be 10 nM, which corresponds to 100 μ g of a 3 kb plasmid.

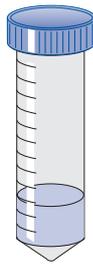
EasyXpress Large-Scale Procedure



Initial in vitro protein synthesis reaction in 10 ml tube



Removal of low-molecular-weight inhibitors by gel filtration

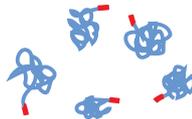


Add Feeding Solution and Energy Mix

Second in vitro protein synthesis reaction in 50 ml tube



Purification, e.g., using Ni-NTA Superflow (not supplied)



Pure recombinant protein

Purification of In Vitro-Synthesized Proteins

In vitro-synthesized proteins that carry a 6xHis tag or *Strep*-tag™ can be easily purified using Ni-NTA Superflow or *Strep*-Tactin™ Superflow respectively. For downstream applications demanding ultrapure protein preparations, proteins carrying both tags (His·*Strep*-tagged proteins) can be purified using a Ni-NTA matrix followed by a second purification using a *Strep*-Tactin matrix. For purification protocols, see page 31.

Purification of tagged proteins using the 6xHis-tag–Ni-NTA interaction

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 (6, 7). Figure 1 shows SDS-PAGE analysis of column fractions obtained from the purification of two 6xHis-tagged proteins, EF-Ts and hTNF-alpha, which were expressed using the EasyXpress Protein Synthesis Mega Kit.

Purification using the *Strep*-tag–*Strep*-Tactin interaction

The *Strep*-tag allows affinity chromatography on immobilized *Strep*-Tactin under physiological conditions. *Strep*-tag II is a short peptide (8 amino acids, WSHPQFEK), which binds with high selectivity to *Strep*-Tactin, an engineered streptavidin. The binding affinity of the *Strep*-tag to *Strep*-Tactin ($K_d = 1 \mu\text{M}$) is nearly 100 times higher than to streptavidin. After a short washing step, gentle elution of purified recombinant protein is performed by addition of low concentrations (2.5 mM) of biotin or desthiobiotin. Desthiobiotin is an inexpensive, reversibly binding, and stable analog of biotin, the natural ligand of streptavidin.

Two-step affinity purification of His·*Strep*-tagged proteins

The initial step of His·*Strep*-tagged protein purification is based on the proven 6xHis-tag Ni-NTA interaction. After elution from a Ni-NTA matrix using imidazole, recombinant proteins (which also carry the *Strep*-tag II epitope) are loaded directly onto a *Strep*-Tactin Matrix. Protein is eluted from the *Strep*-Tactin matrix using either biotin or desthiobiotin. This two-step affinity purification delivers ultrapure (>98% pure) protein.

Efficient Large-Scale Protein Synthesis

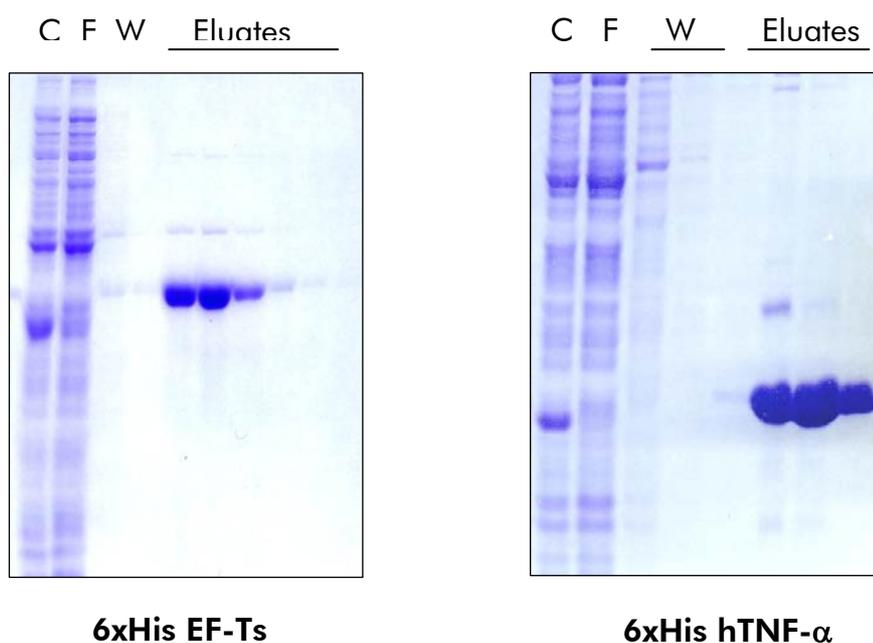


Figure 1 6xHis-tagged EF-Ts and human TNF-alpha were synthesized using the EasyXpress Protein Synthesis Mega Kit and purified under native conditions from the second synthesis reaction using Ni-NTA Superflow. EF-Ts and TNF- α were synthesized using the pIX 2.0 and TAGZyme pQE-2 vectors respectively. Protein was visualized using Coomassie[®] Stain. **C**: Crude lysate; **F**: Flow-through fraction; **W**: Wash fractions.

Protocol: Large-Scale Protein Synthesis Using the EasyXpress Protein Synthesis Mega Kit

This protocol is suitable for the large-scale *in vitro* production of unlabeled recombinant proteins (using the supplied methionine solution) from plasmid DNA in a two-stage procedure. It can also be used for production of selenomethionine-labeled proteins (selenomethionine must be supplied by the user, see below).

Materials and reagents to be supplied by the user

- Plasmid DNA expression template encoding the protein of interest. The plasmid must contain a T7 or other strong *E. coli* promoter and a ribosome binding site (see page 12)
- Optional: Selenomethionine (e.g., Sigma cat. no. S3132)
- Shaking water bath
- Optional: QIArack (cat. no. 19015)

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.
- *E. coli* extracts are provided as 2 individual aliquots in single tubes. Once thawed, use *E. coli* extract within 4 hours.
- Except for the actual transcription-translation incubation and the recycling procedure with the gel filtration column, all handling steps should be carried out on ice.
- The recommended incubation temperature for protein synthesis is 37°C, but lower incubation temperatures may improve protein solubility in some cases.
- For protein synthesis reactions, it is important to follow the order of addition for each component given in the protocol and tables.
- Do not use glass pipets at any stage of the procedure.

Procedure

Initial *in vitro* synthesis reaction

1. **Thaw and store *E. coli* Extract (Mega), Methionine, Feeding Solution (Mega), and Energy Mix on ice. Thaw RNase-free Water and Equilibration/Elution Buffer at room temperature (15–25°C).**

2. **Thaw Reaction Buffer (–Methionine) in the supplied 12 ml plastic tube on ice and vortex thoroughly.**
3. **Add 100 μ l of a 60 mM solution of Methionine (green screw-cap) or selenomethionine (not supplied) to the Reaction Buffer in the 12 ml plastic tube.**

The 12 ml tube will serve as the reaction vessel for the initial protein synthesis reaction.

4. **Add 50 pmol of plasmid DNA expression template to the Reaction Buffer.**

This corresponds to a final concentration of 10 nM (100 μ g of a 3 kb plasmid) in the final 5 ml reaction volume.

5. **Make up the reaction volume to 3.25 ml with RNase-free Water.**

Use the pipetting scheme in Table 1 to calculate the required volume. It is important to follow the order of addition given in the table.

6. **Add 1.75 ml *E. coli* Extract (Mega) to the reaction.**

Important: Do not use a glass pipet to transfer the *E. coli* extract as reaction components may adhere to the glass surface.

7. **Gently mix the reaction by pipetting up and down.**

8. **Incubate the reaction in a water-bath at 37°C with gentle shaking for 1 h.**

Table 1. Initial Protein Synthesis Reaction Components

Reagent	Volume
Reaction Buffer (–Methionine)	1.9 ml
60 mM Methionine or selenomethionine	100 μ l
Plasmid DNA	varies
RNase-free Water	varies
<i>E. coli</i> Extract (Mega)	1.75 ml
Total	5 ml

Recycling the components of the initial synthesis reaction

Steps 9–13 can be performed at room temperature (15–25°C).

- 9. During the protein synthesis reaction, prepare and equilibrate a Gel Filtration Column. Unscrew and remove the bottom closure and peel off the top seal. Allow the storage buffer to drain out. Equilibrate the column by applying 3 x 17 ml aliquots of Equilibration Buffer and allowing the buffer to flow through the column.**

The column can be placed in a QIArack during equilibration.

- 10. After 1 h incubation (step 8), centrifuge the tube containing the protein synthesis reaction at 10,000 x g for 3 min.**

This centrifugation separates precipitates and insoluble target protein.

- 11. Carefully pipet the entire supernatant from step 10 onto the equilibrated Gel Filtration Column.**

Important: Do not use a glass pipet to transfer the supernatant as reaction components may adhere to the glass surface.

- 12. After the supernatant has entered the column, pipet 1 ml Equilibration/Elution Buffer onto the column. Discard the flow-through fraction.**

- 13. Place a 50 ml Reaction Flask (supplied) under the column and pipet 7 ml Equilibration/Elution Buffer onto the column. Collect the flow-through fraction in the Reaction Flask.**

This reaction flask will serve as the reaction vessel for the second protein synthesis reaction. The flow-through fraction contains the recycled high-molecular-weight reaction components.

Second in vitro synthesis reaction

14. Add 200 μ l of a 60 mM solution of Methionine (green screw-cap) or selenomethionine (not supplied) to the protein synthesis reaction (flow-through fraction from step 13).

15. Thoroughly vortex the tube containing Feeding Solution (Mega) and add 1700 μ l to the protein synthesis reaction.

There may be a precipitate visible in the tube containing Feeding Solution. This will not adversely affect the reaction.

16. Add 1100 μ l Energy Mix (red screw-cap) to the protein synthesis reaction.

17. Gently mix the reaction by pipetting up and down.

Table 2 summarizes the components of the second synthesis reaction. It is important to follow the order of addition given in the table.

18. Incubate the reaction in a water-bath at 37°C with gentle shaking for 1 h.

Table 2. Second protein Synthesis Reaction Components

Reagent	Volume
Eluate from gel filtration column	7 ml
60 mM Methionine or selenomethionine	200 μ l
Feeding Solution (Mega)	1.7 ml
Energy Mix	1.1 ml
Total	10 ml

Protocol: Large-Scale Protein Synthesis Using the EasyXpress NMR Protein Synthesis Kit

This protocol is suitable for the *in vitro* production of recombinant isotopically labeled proteins from plasmid DNA using the EasyXpress NMR Protein Synthesis Kit in a two-stage procedure. Proteins are labeled by the addition of isotopically labeled amino acids to the protein synthesis reaction. The supplied Amino Acid Mix (NMR) contains all required amino acids except threonine, arginine, valine, serine, and lysine. These amino acids are supplied individually and can therefore be added to the master mix in a labeled or unlabeled form.

For large-scale protein synthesis using other single amino acid substitution kits (EasyXpress NMR Protein Synthesis Kit – X, where X = Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Leu, Met, Asn, Pro, Gln, Trp, or Tyr) use the protocol on page 26.

Materials and reagents to be supplied by the user

- Plasmid expression DNA template encoding the protein of interest. The plasmid must contain a T7 or strong *E. coli* promoter and a ribosome binding site (see page 12)
- Isotopically labeled threonine, arginine, valine, serine, or lysine
- Shaking water bath
- Optional: QIArack (cat. no. 19015)

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.
- *E. coli* extracts are provided as 2 individual aliquots in single tubes. Once thawed, use *E. coli* extract within 4 hours.
- Except for the actual transcription-translation incubation and the recycling procedure with the gel filtration column, all handling steps should be carried out on ice.
- The recommended incubation temperature for protein synthesis is 37°C, but lower incubation temperatures may improve protein solubility in some cases.
- For protein synthesis reactions, it is important to follow the order of addition for each component given in the protocol and tables.
- Do not use glass pipets at any stage of the procedure.

Procedure

Preparation of amino acid mix

1. Thaw Amino Acid Mix (NMR) and the individual amino acids that will form the master mix.
2. Dissolve isotopically labeled amino acids in RNase-free water to give a concentration of 240 mM.
3. Add 85 μ l of each missing amino acid (labeled or unlabeled) to each vial of Amino Acid Mix (NMR).

Table 3 shows a pipetting scheme for a typical master mix.

Table 3. Amino Acid Master Mixes for Unlabeled and ^{13}C Threonine Labeled Protein Synthesis Reactions

Reagent	Unlabeled reaction	Isotopically labeled reaction
Amino Acid Mix (NMR)	1.275 ml	1.275 ml
Serine (240 mM)	85 μ l	85 μ l
Arginine (240 mM)	85 μ l	85 μ l
Valine (240 mM)	85 μ l	85 μ l
Lysine (240 mM)	85 μ l	85 μ l
Threonine (240 mM)	85 μ l	–
^{13}C labeled threonine (240 mM)	–	85 μ l
Total	1.7 ml	1.7 ml

Initial in vitro synthesis reaction

4. Thaw and store *E. coli* Extract (NMR), Feeding Solution (NMR), and Energy Mix on ice. Thaw RNase-free Water and Equilibration/Elution Buffer at room temperature (15–25°C).
5. Thaw Reaction Buffer without amino acids in the supplied 12 ml plastic tube on ice and vortex thoroughly.

The 12 ml tube will serve as the reaction vessel for the initial protein synthesis reaction.

- 6. Thoroughly vortex the amino acid master mix prepared in step 3. Add 500 μ l amino acid master mix to the Reaction Buffer in the 12 ml plastic tube. Store the remaining amino acid master mix on ice.**
There may be some precipitate in the amino acid master mix. This will not affect the performance of the kit.
- 7. Add 50 pmol of plasmid DNA expression template to the reaction.**
This corresponds to a final concentration of 10 nM (100 μ g of a 3 kb plasmid) in the final 5 ml reaction volume.
- 8. Make up the reaction volume to 3.25 ml with RNase-free Water.**
Use the pipetting scheme in Table 4 to calculate the required volume. It is important to follow the order of addition given in the table.
- 9. Add 1.75 ml *E. coli* Extract (NMR) to the reaction.**
Important: Do not use a glass pipet to transfer the *E. coli* extract as reaction components may adhere to the glass surface.
- 10. Gently mix the reaction by pipetting up and down.**
- 11. Incubate the reaction in a water-bath at 37°C with gentle shaking for 1 h.**

Table 4. Initial Protein Synthesis Reaction Components

Reagent	Volume
Reaction Buffer without amino acids	1.9 ml
Amino acid master mix (prepared in step 3)	500 μ l
Plasmid DNA	varies
RNase-free Water	varies
<i>E. coli</i> Extract (NMR)	1.75 ml
Total	5 ml

Recycling the components of the initial synthesis reaction

Steps 12–16 can be performed at room temperature (15–25°C).

- 12. During the protein synthesis reaction, prepare and equilibrate a Gel Filtration Column. Unscrew and remove the bottom closure and peel off the top seal. Allow the storage buffer to drain out. Equilibrate the column by applying 3 x 17 ml aliquots of Equilibration Buffer and allowing the buffer to flow through the column.**

The column can be placed in a QIArack during equilibration.

- 13. After 1 h incubation (step 11), centrifuge the tube containing the protein synthesis reaction at 10,000 x g for 3 min.**

This centrifugation separates precipitates and insoluble target protein.

- 14. Carefully pipet the entire supernatant from step 13 onto the equilibrated Gel Filtration Column.**

Important: Do not use a glass pipet to transfer the supernatant as reaction components may adhere to the glass surface.

- 15. After the supernatant has entered the column, pipet 1 ml Equilibration/Elution Buffer onto the column. Discard the flow-through fraction.**

- 16. Place a 50 ml Reaction Flask (supplied) under the column and pipet 7 ml Equilibration/Elution Buffer onto the column. Collect the flow-through fraction in the Reaction Flask.**

This reaction flask will serve as the reaction vessel for the second protein synthesis reaction. The flow-through fraction contains the recycled high-molecular-weight reaction components.

Second in vitro synthesis reaction

17. Thoroughly vortex the amino acid master mix remaining from step 6. Add 1 ml of the amino acid master mix to the protein synthesis reaction (flow-through fraction from step 16).

There may be a precipitate visible in the tube containing amino acid master mix. This will not adversely affect the reaction.

18. Vortex the tube containing Feeding Solution (NMR) and add 900 μ l to the protein synthesis reaction.

19. Add 1100 μ l Energy Mix (red screw-cap) to the protein synthesis reaction.

20. Gently mix the reaction by pipetting up and down.

Table 5 summarizes the components of the second synthesis reaction. It is important to follow the order of addition given in the table.

21. Incubate the reaction in a water-bath at 37°C with gentle shaking for 1 h.

Table 5. Second protein Synthesis Reaction Components

Reagent	Volume
Eluate from gel filtration column	7 ml
Amino acid master mix (prepared in step 3)	1 ml
Feeding Mix (NMR)	0.9 ml
Energy Mix	1.1 ml
Total	10 ml

Protocol: Large-Scale Protein Synthesis Using EasyXpress Single Amino Acid Substitution NMR Kits

This protocol is suitable for the in vitro production of recombinant isotopically labeled proteins from plasmid DNA using the EasyXpress NMR Protein Synthesis Kits – X (where X = Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Leu, Met, Asn, Pro, Gln, Trp, or Tyr). Proteins are labeled by the addition of isotopically labeled amino acids to the protein synthesis reaction. The supplied Amino Acid Mix (NMR) contains all required amino acids except that indicated by the kit's name. Isotopically labeled versions of these amino acids must be supplied by the user.

For large-scale protein synthesis using the EasyXpress NMR Protein Synthesis Kit (cat. no. 32526) use the protocol on page 21.

Materials and reagents to be supplied by the user

- Plasmid expression DNA template encoding the protein of interest. The plasmid must contain a T7 or strong *E. coli* promoter and a ribosome binding site (see page 12)
- Isotopically labeled amino acid
- Shaking water bath
- Optional: QIArack (cat. no. 19015)

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.
- *E. coli* extracts are provided as 2 individual aliquots in single tubes. Once thawed, use *E. coli* extract within 4 hours.
- Except for the actual transcription-translation incubation and the recycling procedure with the gel filtration column, all handling steps should be carried out on ice.
- The recommended incubation temperature for protein synthesis is 37°C, but lower incubation temperatures may improve protein solubility in some cases.
- For protein synthesis reactions, it is important to follow the order of addition for each component given in the protocol and tables.
- Do not use glass pipets at any stage of the procedure.

Procedure

Preparation of amino acid mix

1. **Thaw Amino Acid Mix (without indicated amino acid).**
2. **Dissolve isotopically labeled amino acid in RNase-free water to give a concentration of 48 mM.**

Due to the low solubility of some amino acids, the solution may remain cloudy. This will not affect the overall performance of the reaction.

3. **Carefully vortex the isotopically labeled amino acid solution and pipet 425 μ l into each vial of Amino Acid Mix thawed in step 1.**

Table 6 shows a pipetting scheme for a typical master mix prepare with isotopically labeled tyrosine.

Table 6. Amino Acid Master Mix for ^{13}C Tyrosine Labeled Protein Synthesis Reaction

Reagent	Volume
Amino Acid Mix (w/o Tyr)	1.275 ml
^{13}C -labeled tyrosine (48 mM)	425 μ l
Total	1.7 ml

Initial in vitro synthesis reaction

4. **Thaw and store *E. coli* Extract (NMR), Feeding Solution (NMR), and Energy Mix on ice. Thaw RNase-free Water and Equilibration/Elution Buffer at room temperature (15–25°C).**
5. **Thaw Reaction Buffer without amino acids in the supplied 12 ml plastic tube on ice and vortex thoroughly.**

The 12 ml tube will serve as the reaction vessel for the initial protein synthesis reaction.

- 6. Thoroughly vortex the amino acid master mix prepared in step 3. Add 500 μ l amino acid master mix to the Reaction Buffer in the 12 ml plastic tube. Store the remaining master mix on ice.**

There may be some precipitate in the amino acid master mix. This will not affect the performance of the kit.

- 7. Add 50 pmol of plasmid DNA expression template to the reaction.**

This corresponds to a final concentration of 10 nM (100 μ g of a 3 kb plasmid) in the final 5 ml reaction volume.

- 8. Make up the reaction volume to 3.25 ml with RNase-free Water.**

Use the pipetting scheme in Table 7 to calculate the required volume. It is important to follow the order of addition given in the table.

- 9. Add 1.75 ml *E. coli* Extract (NMR) to the reaction.**

Important: Do not use a glass pipet to transfer the *E. coli* extract as reaction components may adhere to the glass surface.

- 10. Gently mix the reaction by pipetting up and down.**

- 11. Incubate the reaction in a water-bath at 37°C with gentle shaking for 1 h.**

Table 7. Initial Protein Synthesis Reaction Components

Reagent	Volume
Reaction Buffer without amino acids	1.9 ml
Amino acid master mix (prepared in step 3)	500 μ l
Plasmid DNA	varies
RNase-free Water	varies
<i>E. coli</i> Extract (NMR)	1.75 ml
Total	5 ml

Recycling the components of the initial synthesis reaction

Steps 12–16 can be performed at room temperature (15–25°C).

- 12. During the protein synthesis reaction, prepare and equilibrate a Gel Filtration Column. Unscrew and remove the bottom closure and peel off the top seal. Allow the storage buffer to drain out. Equilibrate the column by applying 3 x 17 ml aliquots of Equilibration Buffer and allowing the buffer to flow through the column.**

The column can be placed in a QIArack during equilibration.

- 13. After 1 h incubation (step 11), centrifuge the tube containing the protein synthesis reaction at 10,000 x g for 3 min.**

This centrifugation separates precipitates and insoluble target protein.

- 14. Carefully pipet the entire supernatant from step 13 onto the equilibrated Gel Filtration Column.**

Important: Do not use a glass pipet to transfer the supernatant as reaction components may adhere to the glass surface.

- 15. After the supernatant has entered the column, pipet 1 ml Equilibration/Elution Buffer onto the column. Discard the flow-through fraction.**

- 16. Place a 50 ml Reaction Flask (supplied) under the column and pipet 7 ml Equilibration/Elution Buffer onto the column. Collect the flow-through fraction in the Reaction Flask.**

This reaction flask will serve as the reaction vessel for the second protein synthesis reaction. The flow-through fraction contains the recycled high-molecular-weight reaction components.

Second in vitro synthesis reaction

17. Thoroughly vortex the amino acid master mix remaining from step 6. Add 1 ml of the amino acid master mix to the protein synthesis reaction (flow-through fraction from step 16).

There may be a precipitate visible in the tube containing amino acid master mix. This will not adversely affect the reaction.

18. Vortex the tube containing Feeding Solution (NMR) and add 900 μ l to the protein synthesis reaction.

19. Add 1100 μ l Energy Mix (red screw-cap) to the protein synthesis reaction.

20. Gently mix the reaction by pipetting up and down.

Table 8 summarizes the components of the second synthesis reaction. It is important to follow the order of addition given in the table.

21. Incubate the reaction in a water-bath at 37°C with gentle shaking for 1 h.

Table 8. Second protein Synthesis Reaction Components

Reagent	Volume
Eluate from gel filtration column	7 ml
Amino acid master mix (prepared in step 3)	1 ml
Feeding Mix (NMR)	0.9 ml
Energy Mix	1.1 ml
Total	10 ml

Purifying Affinity-Tagged Proteins

Recombinant proteins carrying a 6xHis- or *Strep*-tag can be efficiently purified in a one-step affinity purification procedure using Ni-NTA or *Strep*-Tactin Superflow respectively. Recombinant proteins carrying both tags (His-*Strep*-tagged proteins) can be purified in a two-step procedure using first Ni-NTA and subsequently *Strep*-Tactin Superflow. Purification can be carried out either at 2–8°C or at room temperature (15–25°C).

Purifying 6xHis-tagged proteins using Ni-NTA Superflow

This protocol can be used for purifying up to 15 mg 6xHis- or His-*Strep*-tagged protein generated in an EasyXpress in vitro protein synthesis reaction.

Materials and reagents to be supplied by the user

- EasyXpress in vitro protein synthesis reaction containing 6xHis-tagged target protein
- Ni-NTA Superflow Column (QIAGEN cat. no. 30622)
- Binding, wash, and elution buffers
- Microcentrifuge tubes for elution fractions
- Optional: QIArack (QIAGEN cat. no. 19015)

Buffer compositions can be found in the appendix, page 39.

Procedure

- 1. Centrifuge the protein synthesis reaction at 10,000 x g for 3 min at 15–25°C to precipitate insoluble material.**
- 2. Break the seal at the outlet of a Ni-NTA Superflow column and remove the screw cap.**

Ni-NTA Superflow Columns should be stored vertically. Before opening, ensure that the Ni-NTA Superflow resin is contained in the lower narrow part of the column. If this is not the case, resuspend the resin by gently shaking the column, and keeping it in an upright position until the resin has settled.

- 3. Place the opened column in a rack and allow the storage buffer to drain out.**

The column will not run dry.

- 4. Equilibrate the column by pipetting 10 ml Buffer NPI-10 onto it and allowing the buffer to drain out.**

- 5. Pipet the supernatant from step 1 into a clean 25 ml tube and add 10 ml Buffer NP-10. Pipet the diluted reaction onto the equilibrated Ni-NTA Superflow Column and allow the buffer to drain out.**

Collect and retain the flow-through fraction for subsequent SDS-PAGE analysis.

- 6. Wash the column by pipetting 10 ml Buffer NPI-20 onto it and allowing the buffer to drain out.**

Collect and retain the wash fraction for subsequent SDS-PAGE analysis.

- 7. Place a clean microcentrifuge tube under the column outlet and pipet 1 ml Buffer NPI-250 onto the column. Collect and label the fraction.**

- 8. Repeat step 7 five times to give six elution fractions.**

- 9. Analyze the flow-through, wash, and elution fractions by SDS-PAGE.**

Purifying *Strep*-tagged proteins using *Strep*-Tactin Superflow

This protocol can be used for purifying up to 5 mg *Strep*- or His-*Strep*-tagged protein generated in an EasyXpress in vitro protein synthesis reaction.

Materials and reagents to be supplied by the user

- EasyXpress in vitro protein synthesis reaction or Ni-NTA Superflow elution fractions containing *Strep*-tagged target protein
- *Strep*-Tactin Superflow (QIAGEN cat. no. 30003)
- Polypropylene Columns (5 ml) (QIAGEN cat. no. 34964)
- Wash and elution buffers
- Microcentrifuge tubes for elution fractions
- Optional: QIArack (QIAGEN cat. no. 19015)

Buffer compositions can be found in the appendix, page 26.

Procedure

1. **Centrifuge the protein synthesis reaction at 10,000 x g for 3 min at 4°C to precipitate insoluble material. If using Ni-NTA Superflow elution fractions, pool the fractions.**
2. **Resuspend 5 ml *Strep*-Tactin Superflow and pour into a 5ml polypropylene column.**
3. **Place the opened column in a rack and allow the storage buffer to drain out.**
The column will not run dry.
4. **Equilibrate the column by pipetting 2 x 5 ml Buffer NP onto it and allowing the buffer to drain out.**
5. **Pipet the supernatant or pooled elution fractions from step 1 onto the equilibrated *Strep*-Tactin Superflow Column and allow the buffer to drain out.**
Collect and retain the flow-through fraction for subsequent SDS-PAGE analysis.
6. **Wash the column by pipetting 2 x 5 ml Buffer NP onto it and allowing the buffer to drain out. Repeat the wash with a further 2 x 5 ml Buffer NP.**
Collect and retain the wash fractions for subsequent SDS-PAGE analysis.
7. **Place a clean microcentrifuge tube under the column outlet and pipet 1.25 ml Buffer NPB onto the column. Collect and label the fraction.**

- 8. Repeat step 7 five times to give six elution fractions.**
- 9. Analyze the flow-through, wash, and elution fractions by SDS-PAGE.**

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 | <p>Check the concentration, integrity, and purity of the DNA template.</p> <p>Before large-scale reactions are performed, we recommended checking DNA quality and functionality and developing optimized reaction conditions using small-scale reactions using the EasyXpress Mini or Maxi kit.</p> <p>The physical make-up of the construct can be optimized using linear expression templates generated using the QIAGEN EasyXpress Linear Template Kit.</p> <p>Prepare high-purity plasmid DNA with QIAGEN plasmid kits.</p> |
| b) DNA template not optimally configured, or error in cloning | <p>Check the sequence. Make sure that the start codon is in the right position for expression. Ensure that the expression plasmid contains a T7 promoter or a strong <i>E. coli</i> promoter and a ribosome binding site. See page 8 for recommendations for suitable expression constructs.</p> |
| c) In vitro transcription or in vitro translation is disrupted by expressed protein | <p>In small-scale reactions using the EasyXpress Mini or Maxi Kit, express the control protein EF-Ts alone and in the presence of the target protein. If expression of EF-Ts is inhibited by the presence of the target protein, it may not be possible to efficiently express the target protein using the EasyXpress Protein Synthesis System.</p> |
| d) Amino acid master mix prepared incorrectly | <p>Ensure that the amino acid master mix contains all 20 amino acids (labeled or unlabeled).</p> |

Comments and suggestions

- e) Rigid secondary structures in the mRNA inhibit initiation of translation
- Perform small-scale control expression reactions using the EasyXpress Mini or Maxi Kit. Include an affinity-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

- a) Poor quality or wrong quantity of DNA template.
- Check the concentration, integrity, and purity of the DNA template.
- Before large-scale reactions are performed, we recommended checking DNA quality and functionality and developing optimized reaction conditions using small-scale reactions using the EasyXpress Mini or Maxi Kit.
- The physical make-up of the construct can be optimized using linear expression templates generated using the QIAGEN EasyXpress Linear Template Kit.
- Prepare high-purity plasmid DNA with QIAGEN plasmid kits.
- In small-scale reactions, determine the optimal amount of DNA template used in the in vitro translation by titration.
- b) Template not optimally configured
- Evaluate the level of expression of soluble protein using the EasyXpress Protein Synthesis Mini Kit.
- Adding an affinity tag to the construct may increase yields and/or solubility (8). Conduct trials to find an optimal expression construct using the EasyXpress Protein Synthesis Mini Kit and EasyXpress Linear Template Kit.
- See page 8 for recommendations for suitable expression constructs.

Comments and suggestions

Sufficient protein expression, but low yield of active protein

- | | |
|--|---|
| a) Incorrect folding of the protein due to dependence on posttranslational modifications | <i>E. coli</i> lysate cannot introduce posttranslational modifications like glycosylation, phosphorylation, or signal-peptide cleavage. |
| b) Cofactors required for activity | Add cofactors to synthesis reaction and/or activity assay. |

Expressed protein is insoluble

Protein forms aggregates	We recommended a 37°C incubation temperature for protein synthesis, but lower incubation temperatures may improve protein solubility.
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Sufficient protein expression, but low yield of active protein

Incorrect folding of the protein due to dependence on posttranslational modifications	<i>E. coli</i> lysate cannot introduce post-translational modifications like glycosylation, phosphorylation, or signal-peptide cleavage.
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Appendix A: Optimization of EasyXpress Small-Scale Reactions

Although the EasyXpress system has been developed to give the highest yields of active and soluble protein, it may be possible to further optimize the synthesis procedure for individual proteins, i.e., the total expression or the solubility may be increased by including additives in the synthesis reaction.

As the response to additives is protein-dependent, no general recommendation can be provided. Conditions that give improved results in small-scale reactions should then be transferred linearly to the large-scale reaction (e.g., final additive concentrations showing a positive effect in small-scale reactions should be maintained in the large-scale reaction). It is important that the total volume of the large-scale reactions does not exceed 5.25 ml (first round of synthesis) and 10.5 ml (second round of synthesis) after addition of additives. The table below gives some examples of reagents and reaction conditions that may be lead to improved results with regard to protein solubility and/or yield.

Reaction condition	Optimization suggestions
Template concentration	Titrate between 0.2 and 2 μg per 50 μl reaction
Presence of IPTG	Usually not required, can be added to 1 mM
Temperature	Reaction can be performed at temperatures from 15–37°C
Presence of detergents*	Detergents can be added to a final concentration of 0.05–1% (v/v)
Presence of cofactors	Add as required

* Some detergents may reduce efficiency of protein expression.

Appendix B: Buffer Compositions

NPI-10 (Ni-NTA Superflow binding buffer, 1 Liter):

50 mM NaH ₂ PO ₄	6.90 g NaH ₂ PO ₄ ·H ₂ O (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)

Adjust pH to 8.0 using NaOH.

NPI-20 (Ni-NTA Superflow wash buffer, 1 Liter):

50 mM NaH ₂ PO ₄	6.90 g NaH ₂ PO ₄ ·H ₂ O (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)

Adjust pH to 8.0 using NaOH.

NPI-250 (Ni-NTA Superflow elution buffer, 1 Liter):

50 mM NaH ₂ PO ₄	6.90 g NaH ₂ PO ₄ ·H ₂ O (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)

Adjust pH to 8.0 using NaOH.

NP (Strep-Tactin Superflow binding and wash buffer, 1 Liter):

50 mM NaH ₂ PO ₄	6.90 g NaH ₂ PO ₄ ·H ₂ O (MW 137.99 g/mol)
300 mM NaCl	17.54 g NaCl (MW 58.44 g/mol)

Adjust pH to 8.0 using NaOH.

NPB (Strep-Tactin Superflow elution buffer, 1 Liter):

50 mM NaH ₂ PO ₄	6.90 g NaH ₂ PO ₄ ·H ₂ O (MW 137.99 g/mol)
300 mM NaCl	17.54 g NaCl (MW 58.44 g/mol)
2.5 mM Desthiobiotin	0.54 g Desthiobiotin (Sigma cat. no. D 1411)

Adjust pH to 8.0 using NaOH.

References

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- 3) Yokoyama, S. (2003). Protein expression systems for structural genomics and proteomics. *Curr. Opin. Chem. Biol.* **7**, 39.
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- 6) Hochuli, E., Dobeli, H., and Schacher, A. (1987). New metal chelate adsorbent selective for proteins and peptides containing neighboring histidine residues. *J. Chromatog.* **411**, 177.
- 7) Ni-NTA — Setting the affinity purification standard, QIAGEN News 2005. 35.
- 8) Zacharias, A., Schäfer, F., Müller, S., and von Groll, U. (2004). Recombinant-protein solubility screening using the EasyXpress in vitro translation system. QIAGEN News 2004 e6.
http://www1.qiagen.com/literature/qiagennews/weeklyArticle/04_02/e6/default.aspx .

Ordering Information

Product	Contents	Cat. no.
EasyXpress Protein Synthesis Mega Kit	For 2 x 5 ml reactions: <i>E. coli</i> extract, reaction buffers, amino acid mix w/o methionine, methionine, RNase-free water, gel-filtration columns, and reaction flasks	32516
EasyXpress NMR Protein Synthesis Kit	For 2 x 5 ml reactions: <i>E. coli</i> 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: <i>E. coli</i> 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: <i>E. coli</i> 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: <i>E. coli</i> 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: <i>E. coli</i> 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: <i>E. coli</i> 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: <i>E. coli</i> extract, reaction buffers, amino acid mix w/o Gln (to be supplied by user), RNase-free water, gel-filtration columns, and reaction flasks	32535

Product	Contents	Cat. no.
EasyXpress NMR Protein Synthesis Kit – G	For 2 x 5 ml reactions: <i>E. coli</i> 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: <i>E. coli</i> extract, reaction buffers, amino acid mix w/o His (to be supplied by user), RNase-free water, gel-filtration columns, and reaction flasks	32537
EasyXpress NMR Protein Synthesis Kit – I	For 2 x 5 ml reactions: <i>E. coli</i> 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: <i>E. coli</i> 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: <i>E. coli</i> 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: <i>E. coli</i> 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: <i>E. coli</i> 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: <i>E. coli</i> 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: <i>E. coli</i> 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.
EasyXpress Protein Synthesis Mini Kit	For 20 x 50 μ l reactions: <i>E. coli</i> extract, reaction buffer, RNase-free water, and positive-control DNA	32502
EasyXpress Protein Synthesis Maxi Kit	For reactions up to 4000 μ l: 4 x 350 μ l <i>E. coli</i> 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
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
<i>Strep</i> -Tactin Superflow (2 ml)*	For batch and HPLC purification of <i>Strep</i> -tagged proteins: 2 ml <i>Strep</i> -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
<i>Strep</i> -Tactin Magnetic Beads (2 x 1 ml)*	For micro-scale purification of <i>Strep</i> -tagged proteins: 2 x 1 ml <i>Strep</i> -Tactin-charged magnetic agarose beads (10% suspension)	36311

* Larger sizes available; please inquire

Product	Contents	Cat. no.
Protein detection		
Penta-His HRP Conjugate Kit	125 μ l Penta-His HRP Conjugate, 5 g Blocking Reagent, 50 ml Blocking Reagent Buffer (10x concentrate)	34460
<i>Strep</i> -tag Antibody (100 μ g)	Mouse monoclonal antibody that recognizes the <i>Strep</i> -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
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

* Larger sizes available; please inquire

Notes

Notes

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Tecnolab S.A.
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Fax: (011) 4553 3331
E-mail: info@tecnolab.com.ar
Web site: www.tecnolab.com.ar

Brazil

Uniscience do Brasil
Tel: 011 3622 2320
Fax: 011 3622 2323
E-mail: info@uniscience.com
Web site: www.uniscience.com

Chile

Biosonda SA
Tel: 562 209 6770
Fax: 562 274 5462
E-mail: ventas@biosonda.cl
Web site: www.biosonda.cl

Croatia

INEL Medicinska Tehnika d.o.o.
Tel: (01) 2984-898
Fax: (01) 6520-966
E-mail: inel-medicinska-tehnika@zg.htnet.hr

Cyprus

Scientronics Ltd
Tel: 02-357 22 765416
Fax: 02-357 22 764614
E-mail: a.sarpetsas@biotronics.com.cy

Czech Republic

BIO-CONSULT spol. s.r.o.
Tel/Fax: (420) 2 417 29 792
E-mail: info@bioconsult.cz
Web site: www.bioconsult.cz

Egypt

Clinilab
Tel: 52 57 212
Fax: 52 57 210
E-mail: Clinilab@link.net

Estonia

PEAI-Est OÜ
Tel: (051) 65 830
Fax: (07) 383 360
E-mail: langel@ut.ee

Greece

BioAnalytica S.A.
Tel: (210)-640 03 18
Fax: (210)-646 27 48
E-mail: bioanalyt@hol.gr
Web site: www.bioanalytica.gr

Hungary

Kasztel-Med Co. Ltd.
Tel: (01) 385 3887
Fax: (01) 381 0695
E-mail: info@kasztel.hu
Web site: www.kasztel.hu

India

Genetix
Tel: (011)-2542 1714
or (011)-2515 9346
Fax: (011)-2546 7637
E-mail: genetix@nda.vsnl.net.in

Israel

Westburg (Israel) Ltd.
Tel: 08-6900655
or 1-800 20 22 20 (toll free)
Fax: 08-6900650
E-mail: info@westburg.co.il
Web site: www.westburg.co.il

Korea

LRS Laboratories, Inc.
Tel: (02) 924-86 97
Fax: (02) 924-86 96
E-mail: webmaster@lrslab.co.kr
Web site: www.lrslab.co.kr

Malaysia

RESEARCH BIOLABS SDN. BHD.
Tel: (603)-8070 3101
Fax: (603)-8070 5101
E-mail: biolabs@tm.net.my
Web site: www.researchbiolabs.com

Mexico

Quimica Valaner S.A. de C.V.
Tel: (55) 55 25 57 25
Fax: (55) 55 25 56 25
E-mail: ventas@valaner.com
Web site: www.valaner.com

New Zealand

Biolab Ltd
Tel: (09) 980 6700
or 0800 933 966
Fax: (09) 980 6788
E-mail: biosciences@nzl.biolabgroup.com
Web site: www.biolabgroup.com/nzl

Poland

Syngen Biotech Sp.z.o.o.
Tel: (071) 798 58 50 - 52
Fax: (071) 798 58 53
E-mail: info@syngen.pl
Web site: www.syngen.pl

Portugal

IZASA PORTUGAL, LDA
Tel: (21) 424 7312
Fax: (21) 417 2674
E-mail: consultasbiotec@izasa.es

Saudi Arabia

Abdulla Fouad Co. Ltd.
Tel: (03) 8324400
Fax: (03) 8346174
E-mail: sadiq.omar@abdulla-fouad.com

Singapore

Research Biolabs Pte Ltd
Tel: 6777 5366
Fax: 6778 5177
E-mail: sales@researchbiolabs.com
Web site: www.researchbiolabs.com

Slovak Republic

BIO-CONSULT Slovakia spol. s.r.o.
Tel/Fax: (02) 5022 1336
E-mail: bio-cons@cdicon.sk
Web site: www.bioconsult.cz

Slovenia

MEDILINE d.o.o.
Tel: (01) 830-80-40
Fax: (01) 830-80-70
or (01) 830-80-63
E-mail: mediline@siol.net

South Africa

Southern Cross Biotechnology (Pty) Ltd
Tel: (021) 671 5166
Fax: (021) 671 7734
E-mail: info@scb.co.za

Spain

IZASA, S.A.
Tel: (93) 902.20.30.90
Fax: (93) 902.22.33.66
E-mail: consultasbiotec@izasa.es

Taiwan

TAIGEN Bioscience Corporation
Tel: (02) 2880 2913
Fax: (02) 2880 2916
E-mail: taigen@ms10.hinet.net

Thailand

Theera Trading Co. Ltd.
Tel: (02) 412-5672
Fax: (02) 412-3244
E-mail: theetrad@samart.co.th

Turkey

Medek Medikal Ürünler ve Sağlık Hizmetleri A. S.
Tel: (216) 302 15 80
Fax: (216) 302 15 88
E-mail: makalp@med-ek.com

All other countries

QIAGEN GmbH, Germany

Australia ■ **QIAGEN Pty Ltd** ■ PO Box 641 ■ DONCASTER VIC 3108

Orders 03-9840-9800 ■ Fax 03-9840-9888 ■ Technical 1-800-243-066

Austria ■ **QIAGEN Vertriebs GmbH** ■ Löwengasse 47 / Top 6 ■ A-1030 Wien

Orders: 0800/28-10-10 ■ Fax: 0800/28-10-19 ■ Technical: 0800/28-10-11

Belgium ■ **QIAGEN Benelux B.V.** ■ Spoorstraat 50 ■ 5911 KJ Venlo ■ The Netherlands

Orders 0800-79612 ■ Fax 0800-79611 ■ Technical 0800-79556

Canada ■ **QIAGEN Inc.** ■ 2800 Argentia Road ■ Unit 7 ■ Mississauga ■ Ontario ■ L5N 8L2

Orders 800-572-9613 ■ Fax 800-713-5951 ■ Technical 800-DNA-PREP (800-362-7737)

China ■ **QIAGEN China Representative Office** ■ Room 2213 ■ POS PLAZA ■ Century Avenue 1600 ■

Pudong ■ Shanghai 200122 ■ Telephone 021-51162555 ■ Fax 021-51162500

France ■ **QIAGEN S.A.** ■ 3 avenue du Canada ■ LP 809 ■ 91974 COURTABOEUF CEDEX

Orders 01-60-920-920 ■ Fax 01-60-920-925 ■ Technical 01-60-920-930

Germany ■ **QIAGEN GmbH** ■ QIAGEN Strasse 1 ■ 40724 Hilden

Orders 02103-29-12000 ■ Fax 02103-29-22000 ■ Technical 02103-29-12400

Italy ■ **QIAGEN S.p.A.** ■ Via Grosio, 10/10 ■ 20151 Milano

Orders 02-33430411 ■ Fax 02-33430426 ■ Technical 02-33430414

Japan ■ **QIAGEN K.K.** ■ Forefront Tower II ■ 13-1, Kachidoki 3 Chome ■ Chuo-ku, Tokyo 104-0054

Telephone 03-5547-0811 ■ Fax 03-5547-0818 ■ Technical 03-5547-0811

Luxembourg ■ **QIAGEN Benelux B.V.** ■ Spoorstraat 50 ■ 5911 KJ Venlo ■ The Netherlands

Orders 8002-2076 ■ Fax 8002-2073 ■ Technical 8002-2067

The Netherlands ■ **QIAGEN Benelux B.V.** ■ Spoorstraat 50 ■ 5911 KJ Venlo

Orders 0800-0229592 ■ Fax 0800-0229593 ■ Technical 0800-0229602

Switzerland ■ **QIAGEN AG** ■ Garstligweg 8 ■ 8634 Hombrechtikon

Orders 055-254-22-11 ■ Fax 055-254-22-13 ■ Technical 055-254-22-12

UK and Ireland ■ **QIAGEN Ltd.** ■ QIAGEN House ■ Fleming Way ■ Crawley ■ West Sussex, RH10 9NQ

Orders 01293-422-911 ■ Fax 01293-422-922 ■ Technical 01293-422-999

USA ■ **QIAGEN Inc.** ■ 27220 Turnberry Lane ■ Suite 200 ■ Valencia ■ CA 91355

Orders 800-426-8157 ■ Fax 800-718-2056 ■ Technical 800-DNA-PREP (800-362-7737)

