

January 2009

EasyXpress[®] Large-Scale Synthesis Handbook

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

EasyXpress Protein Synthesis Mega Kit

EasyXpress NMR Protein Synthesis Kits

EasyXpress NMR Uniform Labeling Kits



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Kit Contents

EasyXpress Protein Synthesis Mega Kit	
Catalog no.	32516
Number of reactions	2 x 5 ml
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

EasyXpress NMR Protein Synthesis Kit	
Catalog no.	32526
Number of reactions	2 x 5 ml or 10 x 1 ml
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 (U-15N) Kit
For 2 x 5 ml or 10 x 1 ml reactions**

Catalog no. 32531

Box 1 of 3 — as cat. no. 32526

Box 2 of 3 — as cat. no. 32526

Box 3 of 3

Amino Acid Mix (U-15N, 97-99 %) (glass bottle); All amino acids labeled 115 mg

EasyXpress NMR (U-15N, U-13C) Kit
For 2 x 5 ml or 10 x 1 ml reactions

Catalog no. 32532

Box 1 of 3 — as cat. no. 32526

Box 2 of 3 — as cat. no. 32526

Box 3 of 3

Amino Acid Mix (U-15N, U-13C, 97-99 %) (glass bottle); All amino acids labeled 115 mg

EasyXpress NMR (U-15N, U-13C, U-D) Kit
For 2 x 5 ml or 10 x 1ml reactions

Catalog no. 32535

Box 1 of 3 — as Cat. no. 32526

Box 2 of 3 — as Cat. no. 32526

Box 3 of 3

Amino Acid Mix (U-15N, U-13C, U-D 97 – 99 %) (glass bottle); All amino acids labeled 115 mg

EasyXpress NMR Protein Synthesis Kit — indicated amino acid
For 2 x 5 ml reactions or 10 x 1 ml reactions

Catalog no.

EasyXpress NMR Protein Synthesis Kit w/o A (Ala) 32530

EasyXpress NMR Protein Synthesis Kit w/o C (Cys) 32533

EasyXpress NMR Protein Synthesis Kit w/o E (Glu) 32534

EasyXpress NMR Protein Synthesis Kit w/o G (Gly) 32536

EasyXpress NMR Protein Synthesis Kit w/o H (His) 32537

EasyXpress NMR Protein Synthesis Kit w/o I (Ile) 32538

EasyXpress NMR Protein Synthesis Kit w/o L (Leu) 32539

EasyXpress NMR Protein Synthesis Kit w/o M (Met)	32540
EasyXpress NMR Protein Synthesis Kit w/o F (Phe)	32541
EasyXpress NMR Protein Synthesis Kit w/o P (Pro)	32542
EasyXpress NMR Protein Synthesis Kit w/o W (Trp)	32543
EasyXpress NMR Protein Synthesis Kit w/o 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); e.g. for 32530: amino acid mix without Alanine	2 x 1.275 ml

Storage

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 are shipped at ambient temperature. Upon arrival, store the boxes according to Table 1. When stored under these conditions and handled correctly, both kits can be stored for at least 1 year without showing any reduction in performance.

Table 1. Storage of EasyXpress Kits

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

Product Use Limitations

EasyXpress Kits are intended for molecular biology applications. These products are neither intended for the diagnosis, prevention, or treatment of a disease, nor have they been validated for such use either alone or in combination with other products. Therefore, the performance characteristics of the products for clinical use (i.e., diagnostic, prognostic, therapeutic, or blood banking) are unknown.

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. Separate conditions apply to QIAGEN scientific instruments, service products, and to products shipped on dry ice. Please inquire for more information.

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 back cover or visit www.qiagen.com).

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 sample and assay technologies 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 see our Technical Support Center at www.qiagen.com/Support or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com).

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/support/MSDS.aspx 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 Quality Management System, each lot of EasyXpress Kits is tested against predetermined specifications to ensure consistent product quality.

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. Large-scale in vitro translation reactions enable the rapid production of large amounts of recombinant proteins that are suitable for a range of downstream applications, including structural analysis. QIAGEN has therefore developed EasyXpress products for large-scale production of up to 10 mg of recombinant protein.

The EasyXpress Protein Synthesis Mega Kit allows rapid production of milligram amounts 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, cytotoxic amino acid selenomethionine. The incorporation of selenomethionine into in vitro synthesized proteins facilitates phasing of crystal protein structures and atomic structure determination using X-ray crystallography (1, 2).

The incorporation of stable-isotope–labeled amino acids into in vitro synthesized proteins allows structural determination in solution by high-resolution nuclear magnetic resonance (NMR) spectroscopy (1, 3, 4). The EasyXpress NMR (U-¹⁵N), (U-¹⁵N, U-¹³C), and (U-¹⁵N, U-¹³C, U-D) Kits allow the cell-free expression of uniformly, stable-isotope labeled proteins with the indicated single, double or triple label. In contrast to in vivo expression in D₂O-based medium, where deuterated proteins are usually expressed with significantly lower yields, cell-free expression of triple-labeled proteins results in consistent or enhanced yields compared to the unlabeled protein (Figure 1). This, coupled with the low amount of labeled amino acids required in cell-free expression in general, allows the application of uniform and amino acid-specific labeling methods in a more efficient and economical manner than for in vivo expression.

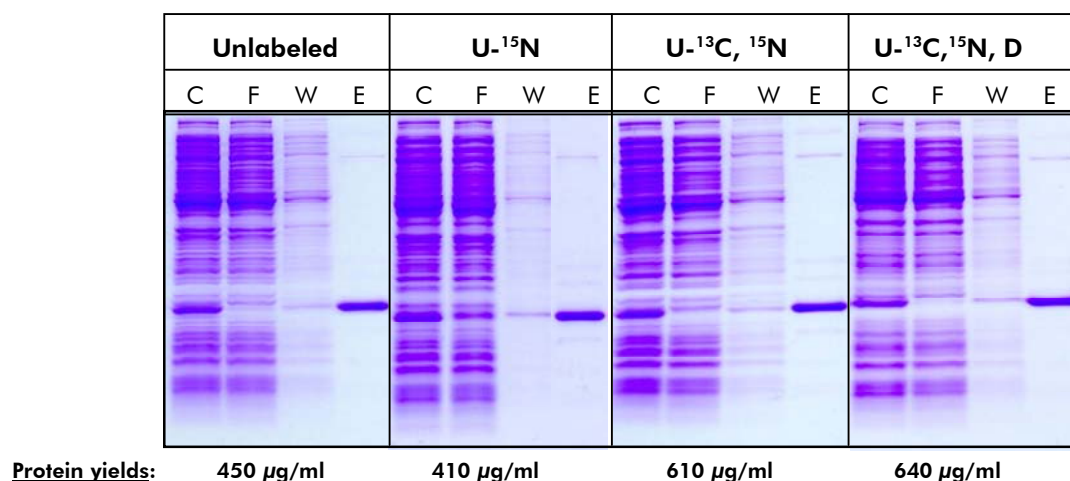


Figure 1. Efficient synthesis and purification of SI-labeled 6xHis-Interleukin-1 β .

Unlabeled and uniformly SI-labeled 6xHis-tagged human Interleukin-1 β samples were synthesized using the EasyXpress NMR Protein Synthesis Kit (1 ml reactions) and purified under native conditions using Ni-NTA Superflow. **C**: Crude lysate; **F**: Flow-through fraction; **W**: Wash fraction; **E**: Eluate. Protein yield was determined by Bradford assay.

Advances in NMR technologies (e.g., Micro- and Cryoprobes) allow the use of lower amounts of labeled protein for NMR data acquisition (5), proteins can optionally be expressed in medium-scale (1 ml) or large-scale (5 ml) EasyXpress NMR Protein Synthesis reactions (Figure 2).

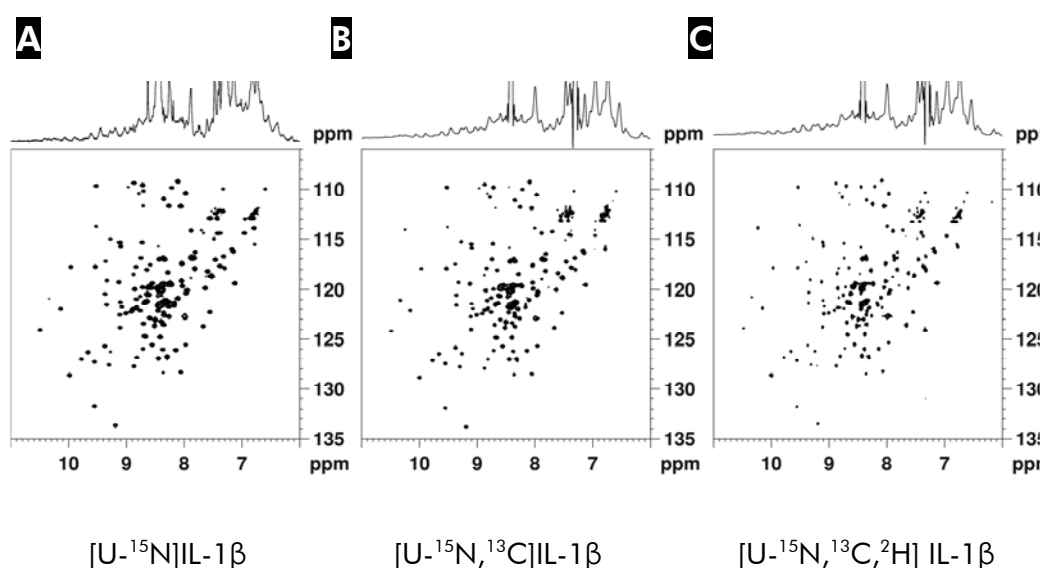


Figure 2. ¹H-¹⁵N HSQC spectra of uniformly SI-labeled 6xHis-tagged human Interleukin-1 β samples synthesized with the EasyXpress NMR Protein Synthesis Kit. **A**

Uniformly ¹⁵N-labeled 6xHis-IL-1 β . **B** Uniformly ¹³C-, ¹⁵N-labeled 6xHis-IL-1 β . **C** Uniformly ¹³C-, ¹⁵N, ²H-labeled 6xHis-IL-1 β . NMR spectra were acquired at 600 MHz using a 1.7 mm H-C/N Micro Cryoprobe from Bruker Biospin.

Triple labeling with ^{15}N , ^{13}C and ^2H is used to facilitate NMR spectroscopy of proteins of above ca. 25 kDa in size. Complete aliphatic ^2H incorporation results in significant reduction of relaxation times of ^{13}C , leading to improved resolution and sensitivity for NMR assignment studies (Figure 3).

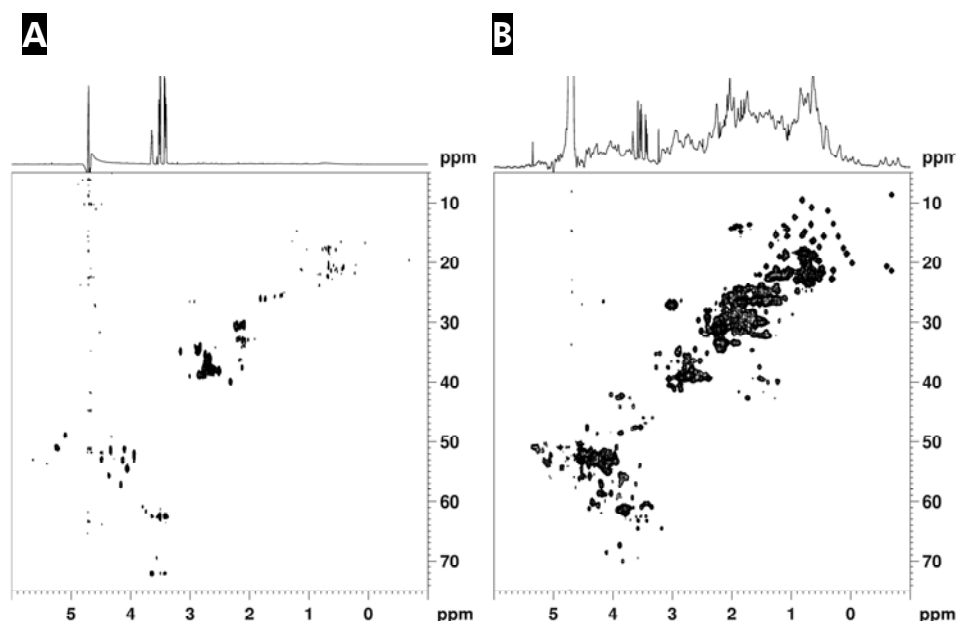


Figure 3. ^1H - ^{13}C HSQC spectra of uniformly SI-labeled 6xHis-tagged human Interleukin-1 β samples synthesized with the EasyXpress NMR Protein Synthesis Kit. **A** Uniformly ^{15}N , ^{13}C , and ^2H -labeled 6xHis-IL-1 β . **B** Uniformly ^{13}C -, ^{15}N -labeled 6xHis-IL-1 β . The virtual absence of signals in spectrum A demonstrates the high level of deuteration. An average deuteration level of 90–95% is achieved. See Appendix C for information regarding back protonation. NMR spectra were acquired at 600 MHz using a 1.7 mm H-C/N Micro Cryoprobe from Bruker Biospin.

The EasyXpress NMR Protein Synthesis Kit allows incorporation of the stable-isotope-labeled amino acids threonine, arginine, valine, serine, or lysine. EasyXpress NMR Kits are also available for incorporation of other amino acids with an isotopic label.

By expressing a protein with all 20 SI-labeled amino acids individually followed by NMR spectroscopic analysis, it was demonstrated that scrambling (distribution of isotopic label to other amino acids due to residual amino acid metabolism) is undetectable upon protein production in the EasyXpress *E. coli* extract for 16 amino acids (6). This is in stark contrast to expression in living cells where clean amino acid-specific labeling is difficult to achieve. Residual metabolizing activity under in vitro expression conditions may be observed in the cases of Gln-, Glu-, Asn-, and Asp-selective labeling, which may lead to a degree of isotope scrambling. The use of inhibitors of the amino acid metabolism, however, can suppress scrambling in these cases (Figure 5, page 52; for more detailed information about scrambling see Appendix B).

The EasyXpress System

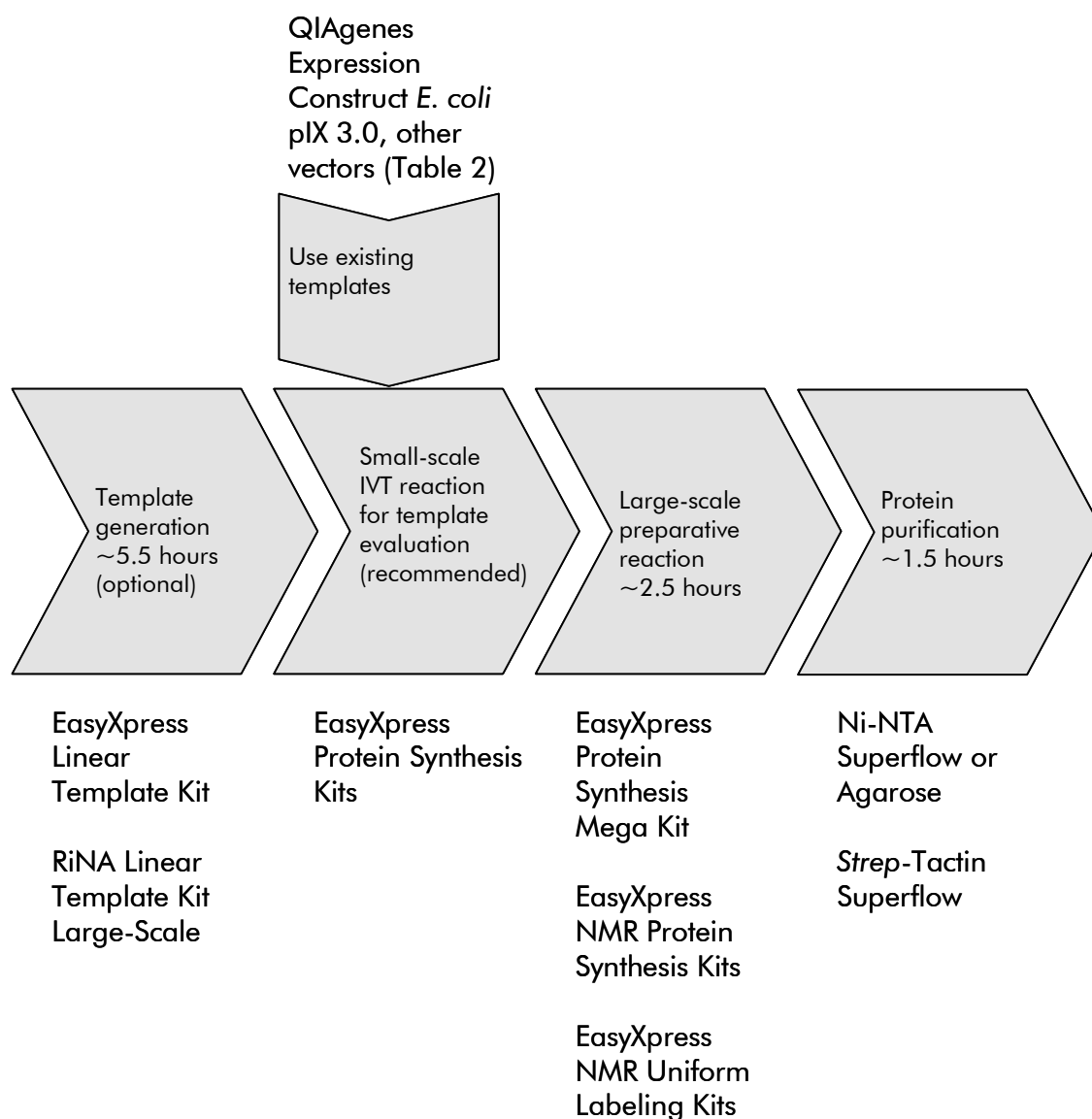
EasyXpress Mega and NMR Kits use highly productive *E. coli* lysates, which contain all translational machinery components (i.e., ribosomes, ribosomal factors, tRNAs, aminoacyl-tRNA synthetases, etc.) as well as T7 RNA polymerase. It is a coupled transcription–translation system that can be used to express full-length proteins from T7 or *E. coli* promoters in a batch reaction using supercoiled DNA templates. The EasyXpress *E. coli* lysates are particularly suitable for expression of stable-isotope labeled proteins since the endogenous amino acids are almost completely depleted.

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 16). 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 10 mg of 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 Kits (cat. no. 32501, 32502, or 32506; see Workflow, page 13). The relative protein yield (mg protein/ml reaction) typically increases by a factor of two 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 and Appendix A).



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 pIX 3.0 or pET3d (Novagen) are a suitable basis for generating expression constructs.

QIAGENes Expression Kits *E. coli* can be used for high-level expression of recombinant proteins in *E. coli* expression systems. QIAGENes Expression Constructs *E. coli* are plasmids containing expression-optimized, synthetic, custom protein coding sequences that are used for in vivo expression in *E. coli* cells or *E. coli*-based cell-free expression systems. (For further information refer to the QIAGENes *E. coli Handbook*.) In most cases, QIAGENes Expression

Constructs show significantly higher expression levels than constructs carrying the wild-type coding sequence.

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 to ensure efficient protein synthesis. Table 2 provides an overview of which vectors have successfully been used with EasyXpress kits.

Table 2. Vectors successfully used with EasyXpress Kits

Vector	Promoter	Protein(s) successfully synthesized
pIX3.0	T7	TFIIA γ , MKK3, HLFA
pET3d	T7	TBP
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
pQE-T7	T7	TNF α
PCR product of RiNA Linear Template Kit Large Scale [‡]	T7	Several different proteins

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

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

[‡] RiNA Linear Template Kit Large Scale, His-Tag or *Strep*-Tag (RiNA, cat. no. C1213-05, C1214-05). Using these specialized PCR products will give similar protein yields (<http://www.rina-gmbh.eu/>).

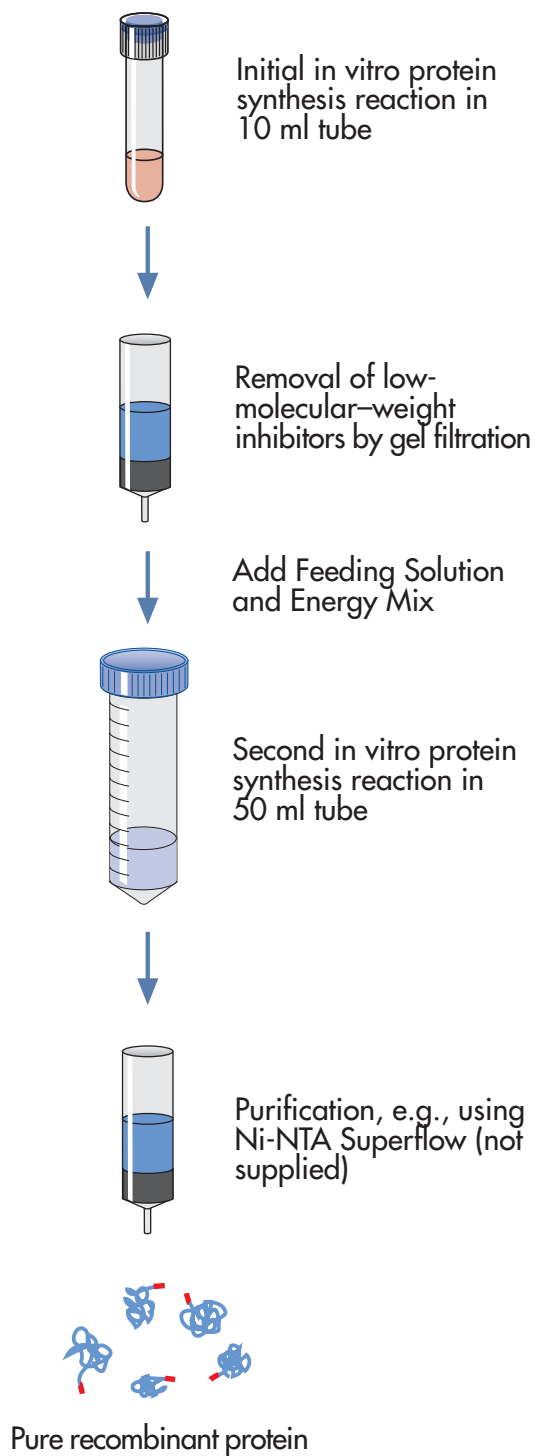
Purification of plasmid DNA expression templates

Greatest protein yields are obtained by 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 (7) 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 in vitro translation reaction should be 10 nM, which corresponds to 20 μ g (1 ml reaction) or 100 μ g (5 ml reaction) of a 3 kb plasmid, respectively.

When using a linear template (page 15), 280 μ l PCR product should be used for a 1 ml reaction (corresponds to approximately 28 μ g) or 1.4 ml for a 5 ml reaction (corresponds to approximately 140 μ g).

EasyXpress Large-Scale Procedure



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 37.

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 (8, 9). Figure 4 shows SDS-PAGE analysis of column fractions obtained from the purification of two 6xHis-tagged proteins, EF-Ts and hTNF- α , 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, WSHHPQFEK), 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.

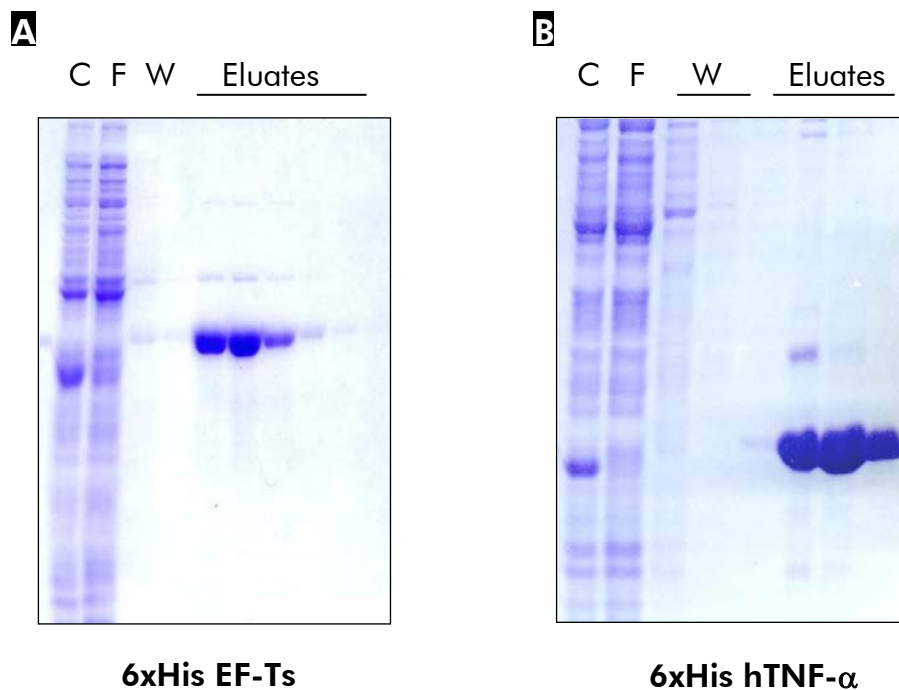


Figure 4. Efficient large-scale protein synthesis and subsequent purification. **A** 6xHis-tagged EF-Ts and **B** human TNF- α were synthesized using the EasyXpress Protein Synthesis Mega Kit and purified under native conditions from the second synthesis reaction using Ni-NTA Superflow. EF-Ts and TNF- 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).

Equipment 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 15)
- **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 if possible use filter pipet tips.
- *E. coli* extracts are provided as two 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 3 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 3. 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. Immediately after starting 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 µl 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 µl 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 µl Energy Mix (red screw-cap) to the protein synthesis reaction.**

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

Table 4 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 4. 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 (5 ml) and Medium-Scale (1 ml) 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 or PCR product 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 Amino Acid Mix (NMR) supplied 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 protein synthesis using other single amino acid substitution kits (EasyXpress NMR Protein Synthesis Kit – X, where X = Ala, Cys, Glu, Gly, His, Ile, Leu, Met, Phe, Pro, Trp, or Tyr), use protocol on page 31.

Recent improvements in NMR technologies (e.g., cryoprobes) allow the use of lower amounts of labeled proteins (5), proteins can be expressed using 1 ml reactions instead of 5 ml reactions. It is advisable to first test and optimize the amount of expressed protein using the EasyXpress Protein Synthesis Kit.

Equipment and reagents to be supplied by the user

- For the 1 ml protocol: NAP-10 columns for recycling step (e.g., GE Healthcare, cat. no. 17-0854-01) and 5 ml plastic tubes
- 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 15)
- Alternatively, PCR product produced with the RiNA Linear Template Kit Large Scale, His-Tag or Strep-Tag (RiNA, cat. no. C1213-05, C1214-05) can be used as template for protein synthesis. This saves time by avoiding cloning procedures. Using these specialized PCR products will give similar protein yields (<http://www.rina-gmbh.eu/>)
- Isotopically labeled threonine, arginine, valine, serine or lysine
- Shaking water bath or thermomixer
- **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 two individual aliquots in single tubes (2 x 5 ml reactions). Once thawed, use *E. coli* extract within 4 hours. If performing 1 ml reactions, all constituents of Box 1 and Box 3 must be vortexed thoroughly and aliquotted in RNase-free reaction tubes (except for the RNase-free water). Do not refreeze and thaw more than twice. Ensure the reaction buffer is carefully aliquotted so that the reaction vial is not overfilled.
- All steps should be carried out on ice, except for the actual transcription–translation incubation and the recycling procedure with the gel filtration column.
- The recommended incubation temperature for protein synthesis is 37°C; however, 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 shown in the protocol and tables.
- Do not use glass pipets at any stage of the procedure.
- ■ denotes values for the EasyXpress NMR Large-Scale protocol (5 ml);
▲ denotes values for the EasyXpress NMR Medium-Scale (1 ml) protocol.

Procedure

Preparation of amino acid mix

1. **Thaw Amino Acid Mix (NMR) and the individual amino acids that will form the master mix. If using the ▲ 1ml protocol, prepare 10 aliquots. Freeze unused aliquots at –20°C or –80°C.**
2. **Dissolve isotopically labeled amino acids in RNase-free water to give a concentration of 240 mM.**
3. **Carefully vortex the isotopically labeled amino acid solution and pipet ■ 85 µl into each vial of Amino Acid Mix thawed in step 1 or ▲ 17 µl to 255 µl of Amino Acid Mix thawed in step 1.**

Table 5 shows a pipetting scheme for a typical master mix.

Table 5. Amino acid master mixes for unlabeled and ¹³C threonine-labeled protein synthesis reactions

Reagent	Unlabeled reaction	Isotopically labeled reaction
	■ / ▲	■ / ▲
Amino Acid Mix (NMR)	1.275 ml / 255 μl	1.275 ml / 255 μl
Serine (240 mM)	85 μl / 17 μl	85 μl / 17 μl
Arginine (240 mM)	85 μl / 17 μl	85 μl / 17 μl
Valine (240 mM)	85 μl / 17 μl	85 μl / 17 μl
Lysine (240 mM)	85 μl / 17 μl	85 μl / 17 μl
Threonine (240 mM)	85 μl / 17 μl	–
¹³ C-labeled threonine (240 mM)	–	85 μl / 17 μl
Total	1.7 ml / 340 μl	1.7 ml / 340 μl

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. If performing the ▲ medium-scale protocol, prepare 10 aliquots of each constituent of Box 1 and Box 3, except for the RNase-free water. Use RNase-free reaction tubes and avoid freezing and thawing the tubes more than twice. Ensure the reaction buffer is carefully aliquotted so that the reaction vial is not overfilled.
6. Thaw Reaction Buffer without amino acids on ice and vortex thoroughly.
 ▲ Transfer 380 μl reaction buffer to a 2 ml Reaction Tube. ■ The 12 ml tube or the ▲ 2 ml Reaction Tube will serve as the reaction vessel for the initial protein synthesis reaction.
7. Thoroughly vortex the amino acid master mix prepared in step 3. Add ■ 500 μl or ▲ 100 μl amino acid master mix to the Reaction Buffer in the ■ 12 ml plastic tube or ▲ 2 ml Reaction 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.

8. Add ■ 50 pmol or ▲ 10 pmol of plasmid DNA expression template to the reaction.

This corresponds to a final concentration of 10 nM (■100 µg / ▲20 µg of a 3 kb plasmid).

PCR product produced using the RiNA Linear Template Kit Large Scale can also be used as a template (see page 25). A modified protocol is provided in the kit handbook.

9. Make up the reaction volume to ■ 3.25 ml or ▲ 650 µl using RNase-free water.

Use the pipetting scheme in Table 6 to calculate the required volume. It is important to follow the order of addition given in the table.

10. Add ■ 1.75 ml or ▲ 350 µl *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.

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

12. Incubate the reaction in a ■ water-bath or ▲ thermomixer at 37°C with gentle shaking for 1 h.

Table 6. Initial protein synthesis reaction components

Reagent	Volume ■ / ▲
Reaction Buffer without amino acids	1.9 ml / 380 µl
Amino acid master mix (prepared in step 3)	500 µl / 100 µl
Plasmid DNA*	Varies
RNase-free water	Varies
<i>E. coli</i> Extract (NMR)	1.75 ml / 350 µl
Total	5 ml / 1 ml

* PCR product produced using the RiNA Linear Template Kit Large Scale can also be used as a template (see page 25). A modified protocol is provided in the kit handbook.

Recycling the components of the initial synthesis reaction

Steps 13–18 can be performed at room temperature (15–25°C).

13. Immediately after starting a protein synthesis reaction, prepare and equilibrate a Gel Filtration Column. Unscrew and remove the bottom closure and top seal. Allow the storage buffer to drain out.

- 14. Equilibrate the column by applying ■ 3 x 17 ml or ▲ 3 x 3 ml aliquots of Equilibration Buffer and allowing the buffer to flow through the column.**

The column can be placed in a QIArack during equilibration.

- 15. After 1 h incubation (step 12), centrifuge the tube containing the protein synthesis reaction at 10,000 x g for 3 min.**

This centrifugation separates precipitates and insoluble target protein.

- 16. Carefully pipet the entire supernatant from step 15 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.

- 17. After the supernatant has entered the column, pipet ■ 1 ml or ▲ 100 µl Equilibration/Elution Buffer onto the column. Discard the flow-through fraction.**

- 18. Place a ■ 50 ml Reaction Flask (supplied) or ▲ a 5 ml plastic tube (not supplied) under the column and pipet ■ 7 ml or ▲ 1.4 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

- 19. Thoroughly vortex the amino acid master mix remaining from step 7. Add ■ 1 ml or ▲ 200 µl of the amino acid master mix to the protein synthesis reaction (flow-through fraction from step 18).**

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

- 20. Vortex the tube containing Feeding Solution (NMR) and add ■ 900 µl or ▲ 180 µl to the protein synthesis reaction.**

- 21. Add ■ 1100 µl or ▲ 220 µl Energy Mix (red screw-cap) to the protein synthesis reaction.**

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

Table 7 summarizes the components of the second synthesis reaction. It is important to follow the order of addition shown in the table.

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

Table 7. Second protein synthesis reaction components

Reagent	Volume ■ / ▲
Eluate from gel filtration column	7 ml / 1.4 ml
Amino acid master mix (prepared in step 3)	1 ml / 200 μ l
Feeding Solution (NMR)	0.9 ml / 180 μ l
Energy Mix	1.1 ml / 220 μ l
Total	10 ml / 2 ml

Protocol: Large-Scale (5 ml) and Medium-Scale (1 ml) 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 or PCR product using the EasyXpress NMR Protein Synthesis Kits – X (where X = Ala, Cys, Glu, Phe, Gly, His, Ile, Leu, Met, Pro, 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 amino acid specific labeling of threonine, lysine, arginine, valine, or serine using the EasyXpress NMR Protein Synthesis Kit (cat. no. 32526) use the protocol on page 25.

Recent improvements in NMR technologies (e.g., cryoprobes) allow the use of lower amounts of labeled proteins (5), proteins can be expressed using 1 ml reactions instead of 5 ml reactions. It is advisable to first test and optimize the amount of expressed protein using the EasyXpress Protein Synthesis Kit.

Equipment and reagents to be supplied by the user

- For the 1 ml protocol: NAP-10 columns for the recycling step (e.g., GE Healthcare, cat. no. 17-0854-01) and 5 ml plastic tubes
- 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 15)
- Alternatively, PCR product produced with the RiNA Linear Template Kit Large Scale, His-Tag or Strep-Tag (RiNA, cat. no. C1213-05, C1214-05) can be used as template for protein synthesis. This saves time by avoiding cloning procedures. Using these specialized PCR products will give similar protein yields (<http://www.rina-gmbh.eu/>)
- Isotopically labeled amino acid
- Shaking water bath or thermomixer
- **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 two individual aliquots in single tubes (2 x 5 ml reactions). Once thawed, use *E. coli* extract within 4 hours. If performing 1 ml reactions, all constituents of Box 1 and Box 3 must be vortexed thoroughly and aliquotted in RNase-free reaction tubes (except for the RNase-free water). Do not refreeze and thaw more than twice. Ensure the reaction buffer is carefully aliquotted so that the reaction vial is not overfilled.
- All steps should be carried out on ice, except for the actual transcription–translation incubation and the recycling procedure with the gel filtration column.
- The recommended incubation temperature for protein synthesis is 37°C; however, 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 shown in the protocol and tables.
- Do not use glass pipets at any stage of the procedure.
- ■ denotes values for the EasyXpress NMR Large-Scale protocol (5 ml);
▲ denotes values for the 1 ml protocol.

Procedure

Preparation of amino acid mix

1. **Thaw Amino Acid Mix (without indicated amino acid). If using the ▲ 1 ml protocol, prepare ten aliquots). Freeze unused aliquots at –20°C or –80°C.**
2. **Dissolve isotopically labeled amino acid in RNase-free water to give a concentration of 48 mM.**
There may be some precipitates in the amino acid master mix. This will not affect the overall performance of the reaction.
3. **Carefully vortex the isotopically labeled amino acid solution and pipet ■ 425 µl into each vial of Amino Acid Mix thawed in step 1 or ▲ 85 µl to 255 µl of Amino Acid Mix thawed in step 1.**

Table 8 shows a pipetting scheme for a typical master mix prepared with isotopically labeled tyrosine.

Table 8. Amino acid master mix for ^{13}C tyrosine-labeled protein synthesis reaction

Reagent	Volume ■ / ▲
Amino Acid Mix (without tyrosine)	1.275 ml / 255 μl
^{13}C -labeled tyrosine (48 mM)	425 μl / 85 μl
Total	1.7 ml / 340 μl

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. If using the medium-scale protocol, prepare 10 aliquots of each constituent of Box 1 and Box 3, except for the RNase-free water. Use RNase-free reaction tubes and avoid freezing and thawing the tubes more than twice. Ensure the reaction buffer is carefully aliquoted so that the reaction vial is not overfilled.

6. Thaw Reaction Buffer without amino acids on ice and vortex thoroughly.

▲ Transfer 380 μl of reaction buffer to a 2 ml Reaction Tube. The ■ 12 ml tube or the ▲ 2 ml Reaction Tube will serve as the reaction vessel for the initial protein synthesis reaction.

7. Thoroughly vortex the amino acid master mix prepared in step 3. Add ■ 500 μl or ▲ 100 μl amino acid master mix to the Reaction Buffer in the ■ 12 ml plastic tube or ▲ 2 ml Reaction 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.

8. Add ■ 50 pmol or ▲ 10 pmol of plasmid DNA expression template to the reaction.

This corresponds to a final concentration of 10 nM (■ 100 μg / ▲ 20 μg of a 3 kb plasmid).

PCR product produced using the RiNA Linear Template Kit Large Scale can also be used as a template (see page 31). A modified protocol is provided in the kit handbook.

9. Make up the reaction volume to ■ 3.25 ml or ▲ 650 µl with RNase-free water.

Use the pipetting scheme in Table 9 to calculate the required volume. It is important to follow the order of addition given in the table.

10. Add ■ 1.75 ml or ▲ 350 µl *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.

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

12. Incubate the reaction in a ■ water-bath or ▲ Thermomixer at 37°C with gentle shaking for 1 h.

Table 9. Initial protein synthesis reaction components

Reagent	Volume ■ / ▲
Reaction Buffer without amino acids	1.9 ml / 380 µl
Amino acid master mix (prepared in step 3)	500 µl / 100 µl
Plasmid DNA*	Varies
RNase-free water	Varies
<i>E. coli</i> Extract (NMR)	1.75 ml / 350 µl
Total	5 ml / 1 ml

* PCR product produced using the RiNA Linear Template Kit Large Scale can also be used as a template (see page 31). A modified protocol is provided in the kit handbook.

Recycling the components of the initial synthesis reaction

Steps 13–18 can be performed at room temperature (15–25°C).

13. Immediately after starting protein synthesis reaction, prepare and equilibrate a Gel Filtration Column. Unscrew and remove the bottom closure and top seal. Allow the storage buffer to drain out.

14. Equilibrate the column by applying ■ 3 x 17 ml or ▲ 3 x 3 ml aliquots of Equilibration Buffer and allowing the buffer to flow through the column.

The column can be placed in a QIArack during equilibration.

15. After 1 h incubation (step 12), centrifuge the tube containing the protein synthesis reaction at 10,000 x g for 3 min.

This centrifugation separates precipitates and insoluble target protein.

16. Carefully pipet the entire supernatant from step 15 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.

17. After the supernatant has entered the column, pipet ■ 1 ml or ▲ 100 μ l Equilibration/Elution Buffer onto the column. Discard the flow-through fraction.

18. Place a ■ 50 ml Reaction Flask (supplied) or ▲ a 5 ml plastic tube (not supplied) under the column and pipet ■ 7 ml or ▲ 1.4 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

19. Thoroughly vortex the amino acid master mix remaining from step 7. Add ■ 1 ml or ▲ 200 μ l amino acid master mix to the protein synthesis reaction (flow-through fraction from step 18).

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

20. Vortex the tube containing Feeding Solution (NMR) and add ■ 900 μ l or ▲ 180 μ l to the protein synthesis reaction.

21. Add ■ 1100 μ l or ▲ 220 μ l Energy Mix (red screw-cap) to the protein synthesis reaction.

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

Table 10 summarizes the components of the second synthesis reaction. It is important to follow the order of addition given in the table.

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

Table 10. Second protein synthesis reaction components

Reagent	Volume ■ / ▲
Eluate from gel filtration column	7 ml / 1.4 ml
Amino acid master mix (prepared in step 3)	1 ml / 200 μ l
Feeding Solution (NMR)	0.9 ml / 180 μ l
Energy Mix	1.1 ml / 220 μ l
Total	10 ml / 2 ml

Protocol: Large-Scale (5 ml) and Medium-Scale (1 ml) Protein Synthesis Using the EasyXpress NMR (U-¹⁵N), (U-¹⁵N, U-¹³C), and (U-¹⁵N, U-¹³C, U-D) Kit

This protocol is suitable for the in vitro production of recombinant, uniformly isotopically labeled proteins from plasmid DNA or PCR product using the EasyXpress NMR Protein Synthesis Kit in a two-stage procedure. Proteins are labeled by the addition of uniformly isotopically labeled amino acids to the protein synthesis reaction. The supplied Amino Acid Mix (NMR) contains all required labeled amino acids U-¹⁵N, U-¹⁵N, and U-¹³C or U-¹⁵N, U-¹³C, and U-D.

Equipment and reagents to be supplied by the user

- For the 1 ml protocol: NAP-10 columns for recycling step (e.g., GE Healthcare, cat. no. 17-0854-01) and 5 ml plastic tubes
- 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 15)
- Alternatively, PCR product produced with the RiNA Linear Template Kit Large Scale, His-Tag or Strep-Tag (RiNA, cat. no. C1213-05, C1214-05) can be used as template for protein synthesis. This saves time by avoiding cloning procedures. Using these specialized PCR products will give similar protein yields (<http://www.rina-gmbh.eu/>)
- Shaking water bath or thermomixer
- **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 two individual aliquots in single tubes (2 x 5 ml reactions). Once thawed, use *E. coli* extract within 4 hours. If performing 1 ml reactions, all constituents of Box 1 and Box 3 must be vortexed thoroughly and aliquotted in RNase-free reaction tubes (except for the RNase-free water). Do not refreeze and thaw more than twice. Ensure the reaction buffer is carefully aliquotted so that the reaction vial is not overfilled.
- All steps should be carried out on ice, except for the actual transcription-translation incubation and the recycling procedure with the gel filtration column.

- The recommended incubation temperature for protein synthesis is 37°C; however, 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 shown in the protocol and tables.
- Do not use glass pipets at any stage of the procedure.
- ■ denotes values for the EasyXpress NMR Large-Scale protocol (5 ml);
▲ denotes values for the 1 ml protocol.

Procedure

Preparation of amino acid mix

1. Dissolve the uniformly labeled Amino Acid Mix ([U-¹⁵N], [U-¹⁵N, U-¹³C] or [U-¹⁵N, U-¹³C, U-D]) in 3050 µl H₂O. If performing the ▲ 1 ml protocol, prepare 10 aliquots. Freeze unused aliquots at –20°C.

Initial in vitro synthesis reaction

2. 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).
3. If performing the medium-scale protocol, prepare 10 aliquots of each constituent of Box 1 and Box 3, except for the RNase-free water. Use RNase-free reaction tubes and avoid freezing and thawing the tubes more than twice. Ensure the reaction buffer is carefully aliquotted so that the reaction vial is not overfilled.
4. Thaw Reaction Buffer without amino acids on ice and vortex thoroughly.
▲ Transfer 380 µl of reaction buffer to a 2 ml Reaction Tube. The ■ 12 ml tube or the ▲ 2 ml Reaction Tube will serve as the reaction vessel for the initial protein synthesis reaction.
5. Thoroughly vortex the amino acid mix prepared in step 1. Add ■ 500 µl or ▲ 100 µl amino acid mix to the Reaction Buffer in the ■ 12 ml plastic tube or ▲ 2 ml Reaction Tube. Store the remaining amino acid mix on ice.

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

6. Add ■ 50 pmol or ▲ 10 pmol of plasmid DNA expression template to the reaction.

This corresponds to a final concentration of 10 nM (■ 100 µg / ▲ 20 µg of a 3 kb plasmid).

PCR product produced using the RiNA Linear Template Kit Large Scale can also be used as a template (see page 37). A modified protocol is provided in the kit handbook.

7. Make up the reaction volume to ■ 3.25 ml or ▲ 650 µl with RNase-free water.

Use the pipetting scheme in Table 11 to calculate the required volume. It is important to follow the order of addition given in the table.

8. Add ■ 1.75 ml or ▲ 350 µl *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.

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

10. Incubate the reaction in ■ a water-bath or ▲ thermomixer at 37°C with gentle shaking for 1 h.

Table 11. Initial protein synthesis reaction components

Reagent	Volume ■ / ▲
Reaction Buffer without amino acids	1.9 ml / 380 µl
Amino acid mix (prepared in step 3)	500 µl / 100 µl
Plasmid DNA*	Varies
RNase-free water	Varies
<i>E. coli</i> Extract (NMR)	1.75 ml / 350 µl
Total	5 ml / 1 ml

* PCR product produced using the RiNA Linear Template Kit Large Scale can also be used as a template (see page 37). A modified protocol is provided in the kit handbook.

Recycling the components of the initial synthesis reaction

Steps 11–16 can be performed at room temperature (15–25°C).

11. Immediately after starting protein synthesis, prepare and equilibrate a Gel Filtration Column. Unscrew and remove the bottom closure and top seal. Allow the storage buffer to drain out.

- 12. Equilibrate the column by applying ■ 3 x 17 ml or ▲ 3 x 3 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 10), 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 or ▲ 100 µl Equilibration/Elution Buffer onto the column. Discard the flow-through fraction.**

- 16. Place a ■ 50 ml Reaction Flask (supplied) or a ▲ 5 ml plastic tube (not supplied) under the column and pipet ■ 7 ml or ▲ 1.4 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 mix remaining from step 5. Add ■ 1 ml or ▲ 200 µl of the amino acid 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 µl or ▲ 180 µl to the protein synthesis reaction.**

- 19. Add ■ 1100 µl or ▲ 220 µl Energy Mix (red screw-cap) to the protein synthesis reaction.**

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

Table 12 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 12. Second protein synthesis reaction components

Reagent	Volume ■ / ▲
Eluate from gel filtration column	7 ml / 1.4 ml
Amino acid master mix (prepared in step 3)	1 ml / 200 μ l
Feeding Solution (NMR)	0.9 ml / 180 μ l
Energy Mix	1.1 ml / 220 μ l
Total	10 ml / 2 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.

Equipment 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 Appendix D

Important point before starting

NMR measurements require low salt and low pH conditions. In contrast to standard protocols, an elution buffer comprising 250 mM instead of 300 mM NaCl and pH 6.5 instead of 8.0 have been successfully used. If NMR measurements require further decreases in NaCl and imidazole concentration and/or pH, protein stability and quantity of elution should be evaluated for a specific protein.

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 NPI-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.

Equipment 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 Appendix D

Important point before starting

NMR measurements require low salt and low pH conditions. In contrast to standard protocols, an elution buffer comprising 250 mM instead of 300 mM NaCl and pH 6.5 instead of 8.0 have been successfully used. If NMR measurements require further decreases in NaCl and imidazole concentration and/or pH, protein stability and quantity of elution should be evaluated for a specific protein.

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. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

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 Protein Synthesis Kits.</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 15 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 Protein Synthesis Kits, 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 | <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> <p>In small-scale reactions, determine the optimal amount of DNA template used in the in vitro translation by titration.</p> |
| b) Template not optimally configured | <p>Evaluate the level of expression of soluble protein using the EasyXpress Protein Synthesis Kits.</p> <p>Adding an affinity tag to the construct may increase yields and/or solubility (10). Conduct trials to find an optimal expression construct using the EasyXpress Protein Synthesis Kits and EasyXpress Linear Template Kit.</p> <p>See page 15 for recommendations for suitable expression constructs.</p> |

Sufficient protein expression, but low yield of active protein

- | | |
|--|---|
| a) Incorrect folding of the protein due to dependence on posttranslational modifications | <p><i>E. coli</i> lysate cannot introduce posttranslational modifications such as glycosylation, phosphorylation, or signal-peptide cleavage.</p> |
|--|---|

Comments and suggestions

- | | |
|------------------------------------|--|
| b) Cofactors required for activity | Add cofactors to synthesis reaction and/or activity assay. |
|------------------------------------|--|

Expressed protein is insoluble

Protein forms aggregates	An incubation temperature of 37°C is recommended for protein synthesis. However, lower incubation temperatures may improve protein solubility.
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Inconsistent data derived from NMR spectra (uniformly labeled proteins)

Partial back protonation of deuterated amino acids	See Appendix C for recommendations.
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Inconsistent data derived from NMR Spectra (amino acid-specific labeling)

Isotope scrambling (Glu labeling)	See Appendix B for more information on inhibition of isotope scrambling.
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Appendix A: Optimization of EasyXpress 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 (e.g., 50 μ l) reactions should be transferred linearly to the large-scale reaction (i.e., final additive concentrations showing a positive effect in small-scale reactions should be maintained in the large-scale reaction). Tables 13 and 14 give some examples of reagents and reaction conditions that may lead to improved results with regard to protein solubility and/or yield.

It may be possible to combine optimized parameters (e.g., incubation at 32°C with 2% glycerol) to further optimize expression, but such enhancements may not always be additive and their effects must be determined empirically.

It is important that the total volume of the large-scale reactions does not exceed 5.35 ml (first round of synthesis) and 10.7 ml (second round of synthesis) after addition of additives.

Table 13. Reaction conditions that may improve results

Reaction condition	Range of conditions evaluated	Recommended starting point for optimization
Temperature	15–37°C	30°C
Incubation time	1–3 h	Usually not required
Reaction dilution	1.1x–1.6x	1.4x
Template concentration	0.2–2 µg DNA per 50 µl reaction	1 µg per 50 µl reaction
Presence of IPTG (for IPTG inducible plasmids)	–	1 mM final concentration
Hydroxy-ectoine	0.1–1 M	0.5 M
D-Sorbitol	0.1–1 M	0.5 M
Glycine Betaine	0.1–1 M	0.5 M
L-Carnitine	0.1–1 M	0.5 M
Detergents*	0.05–1% (v/v)	0.5% (v/v)
Glycerol	Up to 3 % (v/v)	1.5% (v/v)
Presence of cofactors (metal ions)	See Table 14	–
Redox buffer (20x) = Oxidized + Reduced glutathione = 120 mM GssG + 12 mM GSH	0.5x–1.5x	1x
pH adjustment [†]		
Acidic buffer (0.5 M MES, pH 5.5, adjusted with KOH)	Up to 11 µl per 50 µl reaction (pH 6.5–7.4)	–
Basic buffer (0.25 M KOH)	Up to 6 µl per 50 µl reaction (pH 7.4–8.0)	–

* Some detergents may reduce efficiency of protein expression.

[†] Add MES or KOH to EasyXpress Reaction Buffer and reduce volume of water accordingly.

Table 14. Reaction cofactors

Cofactor (salt)	Compatible concentration*	Cofactor (salt)	Compatible concentration)
Ca ²⁺ (CaCl ₂)	20 μ M	Mo ⁶⁺ (Na ₂ MoO ₄)	375 μ M
Co ²⁺ (CoSO ₄)	375 μ M	Se ⁴⁺ (Na ₂ SeO ₃)	1 μ M
Cu ²⁺ (CuCl ₂)	90 μ M	W ⁶⁺ (Na ₂ WO ₄)	1 μ M
Fe ²⁺ (FeSO ₄)	375 μ M	Ni ²⁺ (NiSO ₄)	5 μ M
Mn ²⁺ (MnCl ₂)	45 μ M	Zn ²⁺ (ZnCl ₂)	500 μ M

* The highest concentration tested that had no significant effect on protein synthesis in trials expressing the control protein EF-Ts (His). It is possible that higher concentrations may be added without compromising protein synthesis.

Appendix B: Inhibition of Isotope Scrambling

Using the EasyXpress NMR Protein Synthesis Kit, incorporation of stable-isotope labeled amino acids is both highly efficient and selective. Due to its production procedure, the EasyXpress *E. coli* Extract is far more inert with regard to isotope scrambling than in vivo systems; however, a slight residual metabolizing activity may be observed in the case of Glu-specific labeling. Of note, interconversion between Glu and Asp, as well as between Glu and Gln, may occur to some extent. This can be suppressed by inhibitors of transaminases and glutamine synthase (11).

Table 15 provides an example of transaminase inhibitors and their recommended working concentrations, which helps to prevent isotope scrambling occurring during Glu-specific labeling (Figure 5). It is recommended that protein expression is evaluated in the presence of varying inhibitor concentrations in a small-scale experiment prior to large-scale synthesis.

Table 15. Recommended concentrations of transaminase inhibitors

Selectively labeled amino acid	Recommended inhibitor	Recommended final concentration
Glu	Aminooxyacetate (Sigma, cat. no. C13408)	1. Reaction: 1–2 mM* 2. Reaction: 0.1–1 mM
Glu	L-Methionine sulfoximine† (Sigma, cat. no. M5379)	0.1–0.2 mM in both reactions 1 and 2

* Highest concentration tested with no significant effect on protein synthesis in trials expressing control proteins.

† Inhibitor may reduce efficiency of protein expression.

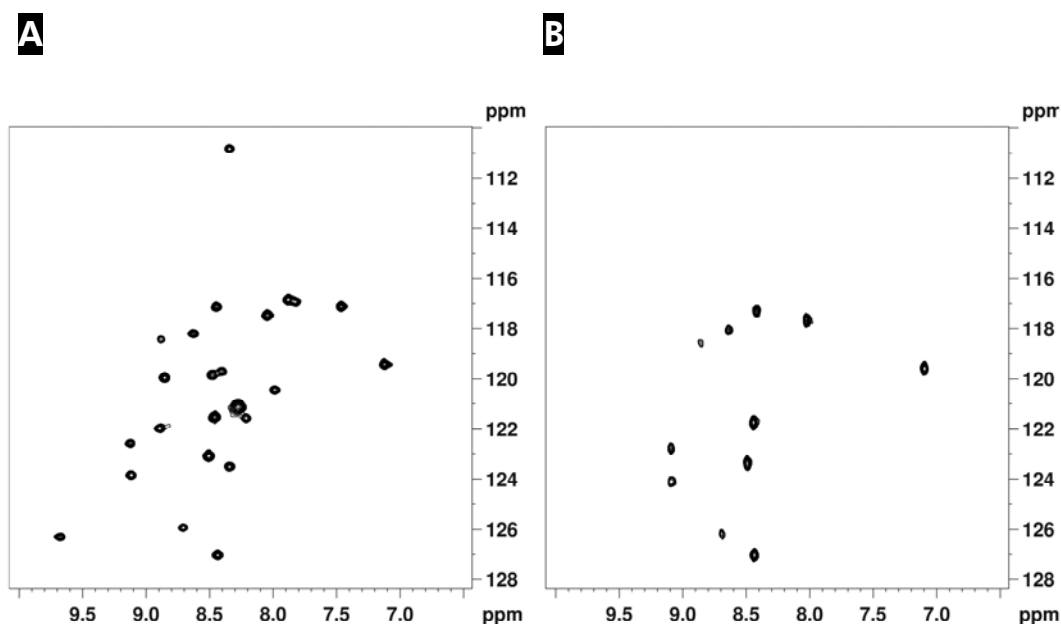


Figure 5. Inhibition of isotopic scrambling. ^1H - ^{15}N HSQC spectra of ^{15}N -Glu selectively labeled IL-1 β synthesized using the EasyXpress NMR Protein Synthesis Kit **A** without scrambling inhibitors **B** in the presence of 2 mM Aminooxyacetate and 0.2 mM L-Methionine sulfoximine. Only signals corresponding to the 11 Glu residues contained in mature IL-1 β are visible in the spectrum when inhibitors are present during protein expression. NMR spectra were acquired at 600 MHz using a 1.7 mm H-C/N Micro Cryoprobe from Bruker Biospin.

Appendix C: Partial Back Protonation of Deuterated Amino Acids

Using the EasyXpress NMR Protein Synthesis Kit with triple-labeled stable-isotope amino acids results in an efficient incorporation level of 90–95% deuterated amino acids, whereas in vivo expression of deuterated proteins can only be achieved after lengthy adaptation of cells to D₂O-based growth media. In contrast, cell-free expression of predeuterated proteins with specifically labeled amino acids results in rapid synthesis and consistent or enhanced yields compared to the unlabeled protein.

Back protonation is characterized as exchange of the H^α and H^β of deuterated amino acids with bulk water and is amino-acid dependent. Asp, Asn, Gln, and Glu show a relatively high degree of partial back protonation. Whereas Thr, His, and Met show only a very low level (12).

To lower partial back protonation reactions (especially amide protons), the triple-labeled amino acid mix can be dissolved in D₂O instead of water. This results in a contingent of 10% deuterium in the reaction volume and decreases back protonation by a factor of approximately two (see Preparation of the amino acid mix, in the protocol on page 38).

For measurements that require a further decrease of cross peaks, it is possible to prepare the Equilibration Buffer in deuterium instead of water. This results in a contingent of 73% deuterium in the reaction volume and further decreases back protonation (see Recycling the components of the initial synthesis reaction, in the protocol on page 39).

Equilibration Buffer (for recycling step)

- 50 mM HEPES, pH 7.6
- 100 mM KOAc
- 50 mM NH₄OAc
- 10 mM MgCl₂
- 0.1 mM EDTA
- 0.002 % NaN₃
- 1 mM DTT
- 10 μM GDP

Appendix D: 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. (For subsequent NMR measurements, QIAGEN recommends 250 mM NaCl (14.61 g), adjust pH to 6.5.)

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. (For subsequent NMR measurements, QIAGEN recommends 250 mM NaCl (14.61 g), adjust pH to 6.5.)

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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 or 10 x 1 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 (U-15N) Kit	For 2 x 5 ml or 10 x 1 ml reactions: <i>E. coli</i> extract, reaction buffers, uniformly 15N labeled amino acid mix, RNase-free water, gel-filtration columns, and reaction flasks	32531
EasyXpress NMR (U-15N, U-13C) Kit	For 2 x 5 ml or 10 x 1 ml reactions: <i>E. coli</i> extract, reaction buffers, uniformly 15N, 13C labeled amino acid mix, RNase-free water, gel-filtration columns, and reaction flasks	32532
EasyXpress NMR (U-15N, U-13C, U-D) Kit	For 2 x 5 ml or 10 x 1 ml reactions: <i>E. coli</i> extract, reaction buffers, uniformly 15N, 13C, and deuterium labeled amino acid mix, RNase-free water, gel-filtration columns, and reaction flasks	32535
EasyXpress NMR Protein Synthesis Kit – A	For 2 x 5 ml or 10 x 1 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 – C	For 2 x 5 ml or 10 x 1 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

Product	Contents	Cat. no.
EasyXpress NMR Protein Synthesis Kit – E	For 2 x 5 ml or 10 x 1 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 – G	For 2 x 5 ml or 10 x 1 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 or 10 x 1 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 or 10 x 1 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 or 10 x 1 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 or 10 x 1 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 or 10 x 1 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

Product	Contents	Cat. no.
EasyXpress NMR Protein Synthesis Kit – P	For 2 x 5 ml or 10 x 1 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 or 10 x 1 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 or 10 x 1 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
EasyXpress Protein Synthesis Kit (5)	For 5 x 50 µl reactions: <i>E. coli</i> extract, reaction buffer, RNase-free water, and positive-control DNA	32501
EasyXpress Protein Synthesis Kit (20)	For 20 x 50 µl 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 µl: 4 x 350 µl <i>E. coli</i> extract, reaction buffer, RNase-free water, and positive-control DNA	32506
EasyXpress Linear Template Kit Plus (20)	For 20 two-step PCRs: For 20 two-step PCRs: HotStar HiFidelity DNA Polymerase, buffer, RNase-free water, Q-Solution, XE-Solution, positive-control DNA, and optimized PCR primers	32723
Protein purification		
Ni-NTA Superflow Cartridges (5 x 1 ml)*	5 cartridges pre-filled with 1 ml Ni-NTA Superflow: for automated purification of His-tagged proteins using liquid chromatography systems	30721

* Larger sizes available; see www.qiagen.com.

Product	Contents	Cat. no.
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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
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
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* Larger sizes available; see www.qiagen.com.

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