



RTS™ 100 Wheat Germ Kit Manual

Eukaryotic cell-free protein synthesis batch screening system based on wheat germ lysate

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For Research Purposes Only. Proteins expressed using the RTS and data derived therefrom that would enable the expression of such proteins (collectively, "Expressed Proteins") may be used only for the internal research of the purchaser of this system. Expressed Proteins may not be sold or transferred to any third party without the written consent of biotechrabbit GmbH.

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The continuous-exchange cell-free (CECF) technology applied in the RTS 100 Wheat Germ CECF, RTS 500 Wheat Germ CECF, RTS 100 *E. coli* Disulfide, RTS 500 *E. coli* Disulfide, RTS 500 ProteoMaster *E. coli* HY and RTS 9000 *E. coli* HY products is based on patented technology (U.S. Patent 5,478,730). The purchase price of this product includes practicing a cell-free expression achieving continuous production of a polypeptide in the presence of a semi-permeable barrier and related processes described in said patents.

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Product Specifications

The RTS 100 Wheat Germ Kit is designed for:

- Efficient expression of up to 5 µg protein per reaction
- Use with non-optimized prokaryotic and eukaryotic T7 promoter-based vectors, as well as with linear templates
- Synthesis of large proteins (264 kDa expressed) with excellent homogeneity
- Protein synthesis in the absence of endotoxins for direct use in cell-based assays
- Rapid parallel screening prior to scaling up to continuous-exchange cell-free (CECF) wheat germ kits

Note: This kit cannot be used for post-translational glycosylation, disulfide-bond formation or signal sequence cleavage.

Product description

The RTS 100 Wheat Germ Kit provides the components and procedures necessary for 24 coupled transcription/translation reactions of 50 µl in batch mode.

Product limitations

The RTS 100 Wheat Germ Kit is 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 the materials described in this text.

Materials supplied

RTS 100 Wheat Germ Kit	Contents and function	No. per kit
Ordering number		BR1402501
Wheat Germ Extract; 100 WG (Vial 1, red screw-cap)	→ Extract from wheat germ → Contains components for transcription and translation	1
Reaction Buffer; 100 WG (Vial 2, blue screw-cap)	→ Substrate mix to prepare reaction solution	1
Energy Mix; 100 WG (Vial 3, green screw-cap)	→ Contains energy components for transcription and translation	1
Control Vector GUS; 100 WG (Vial 4, colorless cap)	→ 50 µg plasmid, lyophilized → Glucuronidase (GUS) expression vector for control reaction	1

Additional materials

To perform the protocols described in this manual, the following additional materials must be provided by the user:

- Eppendorf® ThermoMixer® C (or Eppendorf® Thermomixer Comfort)
- Pipets: 0–10 µl, 10–200 µl and 200–1,000 µl
- Pipet tips autoclaved at 121°C for 20 minutes
- Starting cDNA encoding the protein of interest or vector containing the T7 transcription promoter

For higher protein yields biotechrabbit recommends cloning the cDNA into the RTS pIVEX Wheat Germ His6-tag Vector Set (cat. no. BR1401301) to form a circular template. Alternatively, for initial screening, convert the cDNA into a linear expression template with the RTS Wheat Germ LinTempGen Set, His₆-tag (cat. no. BR1401201). PCR reagents and equipment to either amplify the cDNA for cloning or convert it into a linear expression template will also be required.

Note: The His6-tag is available as an N- or C-terminal version in the RTS pIVEX Wheat Germ His6-tag Vector Set and in the RTS Wheat Germ LinTempGenSet, His6-tag.

- If the reaction is to be performed with an RNA template, biotechrabbit recommends using the SP6/T7 Transcription Kit (Roche) to produce mRNA. A vector containing the cDNA of interest downstream from a suitable promoter (e.g., T3, T7, SP6) will also be required.
- For radioactive labeling (optional) use [³⁵S]-methionine (>1,000 Ci/mmol at 11 mCi/ml) or [¹⁴C]-leucine (> 300 mCi/mmol at 0.1 mCi/ml).
- For reconstitution of the GUS Control Vector, use only deionized DNase- and RNase-free water.

For convenience, additional materials to be supplied by the user are listed at the beginning of the protocol for which they are required.

Shipping and storage conditions

The RTS 100 Wheat Germ Kit is shipped on dry ice.

The RTS 100 Wheat Germ Kit is stable in the dark at –70°C to –80°C until the expiration date printed on the label.

Safety information

All due care and attention should be exercised in the handling of this product. We recommend all users of biotechrabbit products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments or to other applicable guidelines. Specifically, always wear a suitable lab coat, disposable gloves and protective goggles when working with chemicals.

Quality assurance

biotechrabbit products are manufactured using quality chemicals and materials that meet our high standards. All product components are subjected to rigorous quality assurance testing process:

- **Component testing:** each component is tested to ensure the composition and quality meet stated specifications.
- **Performance testing:** each product is tested to ensure it meets the stated performance specification.

Additional quality information is available from www.biotechrabbit.com. Certificate of analysis sheets for biotechrabbit products are available on request.

Product warranty

biotechrabbit is committed to providing products that improve the speed, ease-of-use and quality of enabling technologies.

biotechrabbit 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.

This warranty is in place of any other warranty or guarantee, expressed or implied, instituted by law or otherwise. biotechrabbit provides no other warranties of any kind, expressed or implied, including warranties of merchantability and fitness for a particular purpose. Under no circumstance shall biotechrabbit be responsible for any direct, indirect, consequential or incidental damages or loss arising from the use, misuse, results of use or inability to use its products, even if the possibility of such loss, damage or expense was known by biotechrabbit.

Protocols

Product principle

Introduction

The RTS Wheat Germ Kit is a eukaryotic batch screening system for the rapid synthesis of standard and large proteins (up to 256 kDa expressed) within 2.5 hours. The kit allows the use of a broad range of non-optimized T7 promoter-based expression vectors from prokaryotic and eukaryotic origin in addition to established cell-free wheat germ vectors or linear templates. Using this approach, multiple constructs — including vectors that have not been optimized for wheat germ — can be screened rapidly for expression and functionality in parallel. The procedure saves precious time otherwise used for template generation. The system yields up to 100 µg protein per milliliter reaction, which is sufficient for many downstream applications.

The entire RTS wheat germ system is scalable from µg to mg yields. After screening of synthesis and functionality using the RTS 100 Wheat Germ Kit, batch kit synthesis can be scaled up to mg-scale per reaction using RTS Wheat Germ CECF kits (cat. no. BR1401001 and BR1401101).

Description of procedure

Cell-free transcription and translation take place simultaneously in a reaction tube (Figure 1).

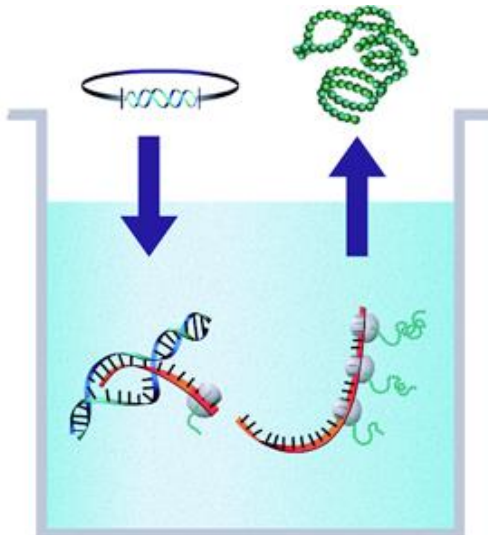


Figure 1. Schematic illustration of the coupled transcription/translation reaction.

An expression plasmid or linear template DNA carrying the gene of interest is added to the reaction tube. In a coupled cell-free reaction, the DNA is first transcribed from the template vector into mRNA by T7 RNA polymerase, followed by translation by the ribosomal machinery present in the wheat germ lysate. Expressed protein accumulates in the reaction tube within 1–2.5 hours.

Template DNA

Any plasmid or linear template – including constructs that have not been optimized for wheat germ – containing the T7 transcription promoter followed by an open reading frame starting with the translation start codon ATG and flanked by up- and downstream untranslated regions, can be used as template for protein synthesis. The usage of non-optimized templates (e.g., vectors designed for expression using *E. coli*) can be used directly without subcloning. Additionally, linear templates produced with the RTS Wheat Germ LinTempGen Set, His6-tag (cat. no. BR1401201) can be used avoiding cloning steps. If maximum yield is required or if scaling up expression, biotechrabbit recommends cloning cDNA into a pIVEX Wheat Germ plasmid (RTS pIVEX Wheat Germ His6-tag Vector Set, cat. no. BR1401301), which contains an enhancer sequence optimized for wheat germ lysates (Figure 2, page 10).

Ready-for-expression ORF clones

As a further option to avoid cloning, ready-for-expression clones such as OmicsLink™ Cell-Free ORF Expression Clones (GeneCopoeia Inc.) can be used. OmicsLink Cell-Free Expression Clones contain various tandem tags and identical 5'- and 3'-regulatory elements as pIVEX Wheat Germ vectors. These expression clones are created by using proprietary high-fidelity non-PCR gene transfer technology that minimizes mutations caused by PCR amplification.

Descriptions of OmicsLink Cell Free Expression Clones, cloning technology and expression data of more than 10,000 human full-length protein coding clones that can be used in the RTS 100 Wheat Germ Kit are available at www.genecopoeia.com.

Coupled cell-free transcription/translation

Similar to other cell-free expression systems, the RTS 100 Wheat Germ system allows expression of exogenously added mRNA. However, it is more convenient to produce the mRNA in a cell-free coupled transcription/translation reaction by starting with a DNA sequence. When DNA template and T7 RNA polymerase are added to the DNA-free wheat germ lysate, transcription and translation are closely coupled in time and space. While the T7 RNA polymerase transcribes the gene of interest encoded by PCR-generated template or vector, the ribosomes provided in the wheat germ lysate begin to translate mRNA.

Note: Yields of batch reactions with the RTS Wheat Germ system can be increased by using CECF format (RTS Wheat Germ CECF, cat. no. BR1401001/BR1401101).

Protocol 1: Preparation of templates for cell-free expression

Cloning into pIVEX Wheat Germ vectors

Any vector or linear DNA to be used in combination with this kit must contain the target gene under control of the T7 promoter. For high yield, synthesis templates should contain 5' and 3' regulatory untranslated regions that are optimized for wheat-germ expression systems (see information included in the RTS pIVEX Wheat Germ His₆-tag Vector Set, cat. no. BR1401301).

Some of the available pIVEX vectors are shown schematically in Figure 2. Sequences and maps of these vectors can be downloaded from www.biotechrabbit.com, cat. no. BR1401301.

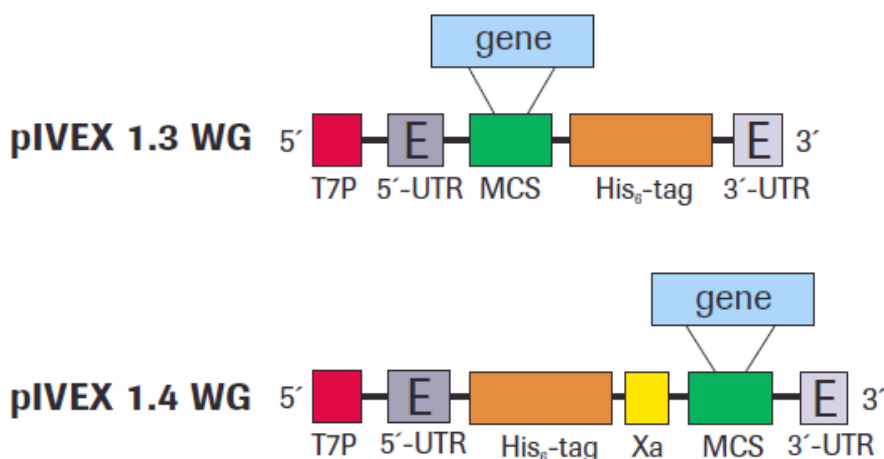


Figure 2. Functional elements of RTS wheat germ expression vectors.

T7P: T7 promoter; **UTR:** untranslated regions containing optimized translation enhancer (E) elements (for details see pack insert for each vector); **His₆-tag:** tag sequence at C- or N-terminal position; **Xa:** Factor Xa restriction protease cleavage site; **MCS:** multiple cloning site in three different reading frames for the insertion of the target gene.

Note: Vector DNA purified from agarose gels cannot be used as template for cell-free expression, because the purification treatment may introduce chemicals that inhibit protein synthesis.

Cloning procedure

Protocols for cloning the cDNA of interest into RTS pIVEX Wheat Germ vectors are provided in the corresponding vector manuals. The protocols are based on either standard restriction digestions, or, for the processing of multiple samples in parallel, In-Fusion® cloning.

Ready-for-expression ORF clones

Ready-for-expression clones (OmicsLink Cell Free ORF Expression Clones) for 20,000 human proteins can also be used (www.genecopoeia.com).

Purity of the plasmid preparation

Plasmids obtained from commercially available DNA preparation kits (e.g., GenUP™ Plasmid Kit, cat. no. BR0700201) are usually sufficiently pure to be used as template in the RTS. If DNA is not sufficiently pure ($A_{260}/A_{280} < 1.7$), use phenol extraction to increase purity and to remove traces of RNase, which may otherwise inhibit template performance in the expression reaction.

Note: Do not purify DNA fragments from agarose gels, since this treatment may inhibit cell-free protein synthesis.

Main applications

For accelerated production of expression templates from wild-type or modified DNA sequences, biotechrabbit offers the option of using PCR instead of subcloning the sequences into vectors. PCR methods permit (for example):

- Addition of the T7 promoter and both the 5'- and 3'-regulatory regions to the cDNA of interest
- Addition of epitope tag sequences to allow detection with antibodies
- Introduction of mutations
- Changes in codon usage
- Construction of truncated proteins

After identifying suitable linear expression templates, template sequences can optionally be subcloned into the RTS pIVEX Wheat Germ vectors. Protocols for cloning the linear expression templates are available in the RTS Wheat Germ LinTempGenSet, His₆-tag Manual (www.biotechrabbit.com, cat. no. BR1401201).

Generation of a linear expression template using a two-step PCR protocol

To incorporate the 5'- and 3'-untranslated regions and the T7 promoter into a non-expressible template, biotechrabbit recommends using a two-step overlap extension PCR protocol, such as the one outlined below. This protocol uses the RTS Wheat Germ LinTempGen Set, His₆-tag (cat. no. BR1401201), which provides DNA containing these regulatory regions, as well as epitope tag sequences.

Equipment and reagents required

- RTS Wheat Germ LinTempGenSet, His₆-tag

Procedure

1. Design a sense primer that contains a 15–20 nucleotide sequence that is identical to the cDNA of interest and an additional 20 base sequence that overlaps the chosen tag region (as indicated in the manual).

Use the RTS Wheat Germ LinTempGen Set, His₆-tag, which allows either the N- or C-terminal epitope tag to be added to the gene of interest.

2. Design an antisense primer that contains a 15–20 nucleotide sequence that is identical to the gene of interest and an additional 20 base sequence that overlaps the chosen tag region (as indicated in the manual).
3. Perform the first PCR in a 50 µl reaction volume, using the primers you designed and template DNA that contains the gene of interest.
4. Perform a second PCR in a 50 µl reaction volume using 2–4 µl (150–300 ng) of the first PCR as template, plus primers and DNA containing the T7 promoter, enhancer elements for translation in wheat germ and C- or N-terminal epitope tags. (This DNA is supplied with the RTS Wheat Germ LinTempGen Set, His₆-tag Kit.)
5. Purify the PCR product using GenUP™ PCR Cleanup Kit (cat. no. BR0700301 or cat. no. BR0700501). Determine the concentration of the PCR product on an agarose gel by comparing to known amounts of DNA molecular size markers. Unpurified PCR products may be used, but these may give lower expression rates.
6. Use 1–2 µg of the PCR product from the second PCR for a 50 µl cell-free protein synthesis reaction.

Note: Do not use agarose gels to purify the PCR product that will be used in the expression reaction, since this purification process may inhibit cell-free protein synthesis.

7. Optional: The desired linear template can be cloned into the RTS pIVEX Wheat Germ vector

Protocol 2: Protein synthesis reaction

Equipment and reagents required

- Eppendorf® ThermoMixer® C (or Eppendorf® Thermomixer Comfort) set to 24°C and a shaking speed of 500 rpm for reaction incubation
- Calibrated pipets
- RNase-free plastic and glassware
- SDS-polyacrylamide gel, to check the control reaction with glucuronidase.

A band with a molecular weight of 68 kDa should be visible after the gel is stained with Coomassie® Brilliant Blue (Figure 3, page 17)

Reagent notes

- Prepare and purify template as detailed in Protocol 1, page 10.
- Do not combine reagents from different kit lots.
- Keep reagents and working solutions on ice until use.
- Store the solutions at –70°C to –80°C.
- Store Bottle 1 (Wheat Germ Lysate) at –80°C after use. The reagent can withstand three freeze–thaw cycles without significant decrease in activity.
- Store the plasmid at –15°C to –25°C or lower temperatures.

Points to consider

Temperature: Optimal temperature for most proteins is 24°C.

Time: Protein synthesis continues for up to 4 hours.

Procedure

1. Set the ThermoMixer to 24°C.
2. Briefly pellet the content of Control Vector GUS tube by centrifugation. Reconstitute the lyophilisate with 65 µl DNase- and RNase-free water.
3. Thaw and store all other kit components quickly using a 25°C water bath. Mix immediately and briefly. Centrifuge briefly to collect the solutions at the bottom of the tubes. Keep reagents on ice until use except the template solution, which should be kept at room temperature.

All solutions should be clear except the Wheat Germ Lysate, which remains cloudy.

4. Prepare reaction solution according to Table 1. Mix briefly after addition of each component. After addition of template, briefly centrifuge the reaction.

Table 1. Reaction solution

- 6.5 µl DNase- and RNase-free water
- 3.5 µl Reaction Buffer
- 20 µl Wheat Germ Extract
- 10 µl Energy Mix
After adding Energy Mix, place the reaction at 20–25°C.
- For the synthesis of the target protein use 10 µl DNase- and RNase-free water containing 7.5 µg circular DNA template or 6.5 µl linear PCR-generated template (approximately 0.5 to 1 µg)
For the positive control reaction use 10 µl Control Vector GUS. For the negative control reaction use 10 µl DNase- and RNase-free Water

Note: For multiple parallel reactions, biotechrabbit recommends starting with the addition of DNase- and RNase-free water containing template DNA to an empty reaction tube. Subsequently, a mixture of DNase- and RNase-free water, Reaction Buffer and Wheat Germ Extract should be prepared and added to the reaction tubes with template. Finally, Energy Mix is added to the mixture containing the template.

5. Start the reaction using the ThermoMixer. Incubate for 2.5 hours at 24°C and 500 rpm.
6. Apply 1 µl of each reaction to a SDS-polyacrylamide gel. Run the gel and stain with Coomassie Brilliant Blue. A band with a molecular weight of approximately 68 kDa should be visible in the reaction with the GUS Control Vector (Figure 3, page 17), but not in the negative control reaction.

Note: The GUS protein can also be detected on a western blot using an anti-His₆-tag antibody.

Improving solubility of the target protein

To reduce aggregation of synthesized proteins — especially membrane proteins — add a mild detergent or a compatible solute, such as 0.05-0.2% Brij 35 or DDM (n-Dodecyl β -D-maltoside) detergent or 0.5 M Sorbitol, to the reaction instead of the corresponding volume of RNase- and DNase-free water.

Radioactive labeling using [³⁵S]-methionine

Procedure

Set up the reaction according to Table 1, page 14, with the following exceptions:

1. Reduce RNase- and DNase-free water to 5.5 μ l.
2. After addition of Reaction Buffer add 1 μ l L-[³⁵S]-methionine 11 mCi/ml.
Note: This labeling is for autoradiographic detection of labeled samples. If using fluorography to detect labeled products, the amount of label can be reduced.
3. After stopping the reaction apply 1–2 μ l of the labeled samples to a SDS-polyacrylamide gel.
4. After the separation, dry the gel and expose it to a screen of a phosphoimager system (3–20 hours exposure time).

Radioactive labeling using [¹⁴C]-leucine

Procedure

Set up the reaction according to Table 1, page 14, with the following exceptions:

1. Reduce RNase- and DNase-free water to 4.5 μ l.
2. After addition of Reaction Buffer add 2 μ l L-[¹⁴C]-leucine 0.1 mCi/ml, >300 mCi/mmol).
3. After stopping the reaction apply 5 μ l of the labeled samples to a SDS-polyacrylamide gel. Refer to "Optimizing SDS-polyacrylamide gel electrophoresis (SDS-PAGE)" on page 16.
4. After the separation, dry the gel and expose it to a screen of a phosphoimager system (3–20 hours exposure time).

Optimizing SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

Precipitation of proteins prior to SDS-PAGE

If reaction volumes of more than 1 μl are loaded on to a SDS gel, the Reaction Solution contains low-molecular-weight-components that interfere with the clear separation of proteins. biotechrabbit recommends that samples are precipitated with acetone prior to the addition of SDS-PAGE sample buffer.

Procedure

Analysis of whole reaction

1. To a 5 μl sample of Reaction Solution, add 50 μl of -20°C cold acetone, mix and incubate on ice for 5 minutes.
2. Centrifuge for 5 minutes at 10,000 rpm.
3. Discard the supernatant and air dry the pellet for 10 minutes. Centrifuging while applying a vacuum may be used.
4. Dissolve the pellet in 20 μl SDS-PAGE sample buffer, heat for 5 minutes at 95°C and apply 5–20 μl to a SDS gel.

Analysis of soluble fraction of the reaction

1. Centrifuge a 5 μl sample of the Reaction Solution for 5 minutes at 10,000 rpm.
2. Pipet the supernatant into a separate tube.
3. Add 50 μl ice-cold acetone to each tube, mix and incubate on ice for 5 minutes.
4. Centrifuge for 5 minutes at 10,000 rpm.
5. Discard the supernatant and air dry the pellet for 10 minutes. Centrifuging while applying a vacuum may be used.
6. Dissolve the pellet in 20 μl SDS-PAGE sample buffer, heat for 5 minutes at 95°C and apply 5–20 μl to a SDS gel.

Supporting Information

Typical results

Standard reaction

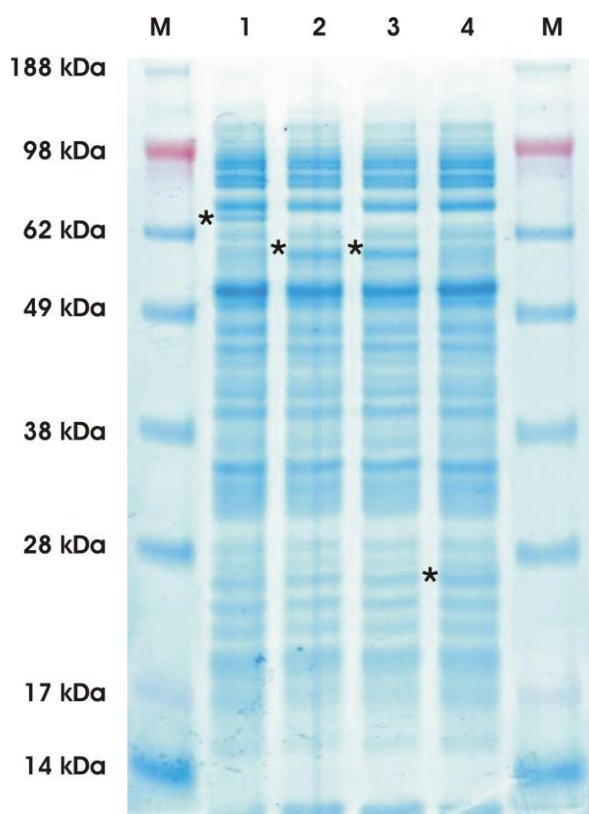


Figure 3. Yields and homogeneity of proteins obtained from vectors of different origin in RTS 100 Wheat Germ expression reactions.

At the completion of the reactions, 0.5 μ l of each reaction mixture was analyzed using SDS-PAGE. Gels were stained with Coomassie Brilliant Blue. Bands of the expected size are marked with an asterisk.

Application: Radioactive labeling with L-[¹⁴C]-leucine

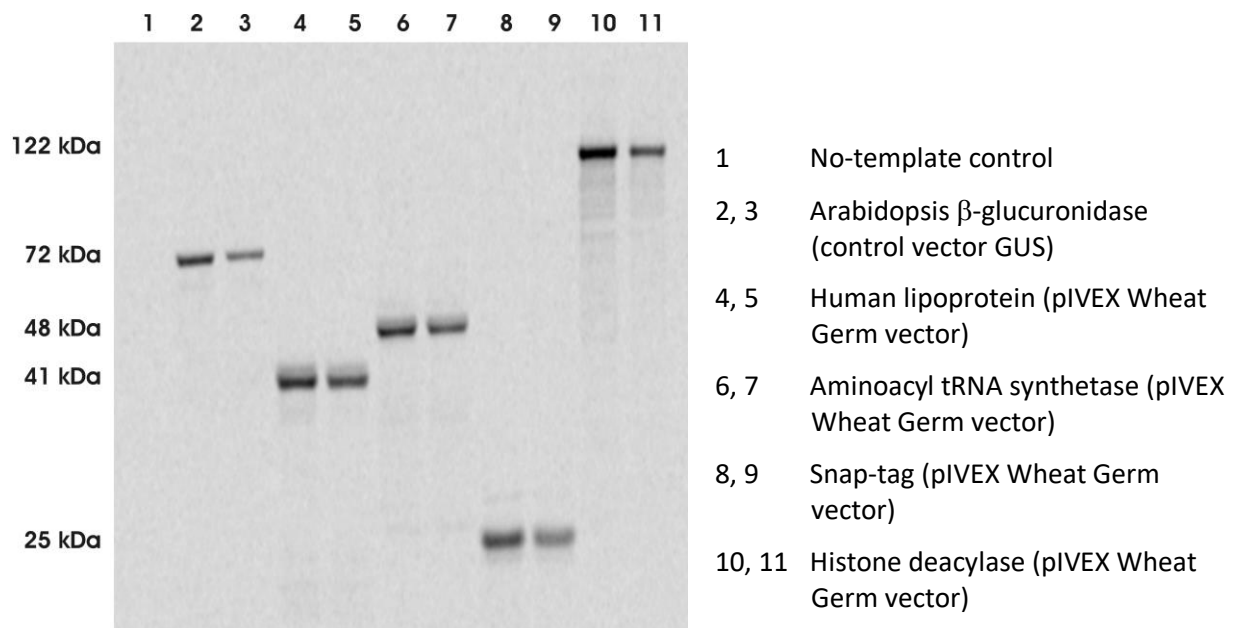


Figure 4. Radioactive labeling reaction.

Radioactive labeling was performed according to procedure on page 15, except the labeled leucine solution contained only 0.5 μ l L-[¹⁴C]-leucine. After labeling, 5 and 2 μ l aliquots from the reactions were applied to a 10% SDS gel. Labeled products were detected by autoradiography after 16 hours exposure using a Typhoon imager (Amersham Pharmacia). Protein quantification was performed by trichloro acetic acid precipitation of 5 μ l samples of radioactively labeled reaction solution followed by scintillation counting. Yields of tested proteins 60–120 μ g/ml.

References

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3. Ausubel, U.K. et al., (1993). *Current Protocols in Molecular Biology*. John Wiley & Sons Inc., New York.
4. Ahmed, A.K. et al., (1975). *J. Biol. Chem.* 250, 8477.
5. Odorzinsky, T.W., Light, A. (1979). *J. Biol. Chem.* 254, 4291.
6. Rudolph, R. et al., (1997). In *Protein Function – A Practical Approach*. Creighton, T. E. ed. Oxford University Press Inc. New York, pp 57–99.

Troubleshooting guide

The following troubleshooting recommendations are designed to address unexpected or undesired results. To ensure optimal use, follow the guidelines and recommendations in the manual.

General problems

Observation	Control protein is not expressed
Possible cause	Kit has not been stored at -15°C to -25°C
Resolving	Order a new kit.
Possible cause	Contamination with RNases
Resolving	Repeat experiment and be sure to work RNase-free at every step.

Problems expressing target protein, although GUS control protein works

Observation	Good protein expression, but low yield of active protein
Possible cause	Incorrect folding of the protein due to a requirement for co-factors or disulfide bond formation
Resolving	Add necessary cofactors. Try the RTS 100 <i>E. coli</i> Disulfide Kit for the expression of disulfide bonded proteins (biotechrabbit). Alternatively, after the reaction, oxidize the protein to form disulfide bonds [4, 5].
Possible cause	Dependence on other secondary modifications
Avoiding	The RTS Wheat Germ Lysate cannot introduce post-translational modifications such as glycosylation, disulfide bond formation or signal sequence cleavage.

Observation	Low expression yield
Possible cause	Expression time too short
Resolving	Extend expression time.
Possible cause	Epitope tag interferes with protein folding
Resolving	Use different pIVEX WG vectors to prepare the expression template. Try different epitope tag sequences that are introduced via PCR.
Possible cause	Amount of template DNA not optimal
Resolving	For optimal results, vary the DNA concentration in a 50 µl reaction between 5 µg and 10 µg for circular templates or between 0.2 µg and 2 µg for linear templates.

Observation	Several product bands on SDS-PAGE or product smaller than expected
Possible cause	Proteolytic degradation
Resolving	Add protease inhibitors to reaction. For example, dissolve 1 tablet of Complete Mini, EDTA-free (Roche) in 0.5 ml nuclease-free water and add 2 µl of this solution to a 50 µl reaction mix.
Possible cause	Internal initiation site
Resolving	Eliminate the corresponding methionine by point mutation.
Possible cause	Premature termination of translation
Resolving	Check sequence of target gene for incorrect reading frame or mutation that produces a stop codon. Search for strong secondary structures in the mRNA and eliminate them by using conservative mutations.

Observation	Product in the pellet fraction
Possible cause	Aggregation
Resolving	Add/adjust chaperones. Adjust experimental conditions (time, temperature). Add mild detergents (e.g., up to 0.05% [v/v] Brij 35, Digitonin or Nonidet NP-40 or up to 0.1% Triton® X-100 [v/v] or 0.1% CHAPS for membrane proteins)

Observation	No expression of the target gene, but normal expression of GUS
Possible cause	Cloning error
Resolving	Check the sequence
Possible cause	Low purity of DNA template
Resolving	Ensure the absorbance ratio 260 nm/280 nm is at least 1.7. Perform a phenol extraction if purity is low. Make a new template preparation.
Possible cause	Contamination with RNases
Resolving	Repeat experiment and be sure to work RNase-free at every step.
Possible cause	No initiation of translation due to strong secondary structures of the mRNA
Resolving	Use different pIVEX WG vectors to prepare the expression template. Try to express the protein as an N-terminally tagged protein (e.g., using pIVEX 1.4 WG).
Possible cause	Expressed protein interferes with the translation or transcription process
Resolving	Express gene of interest together with GUS. If GUS expression is inhibited, the target protein cannot be expressed with the kit.

Ordering Information

Product	Size	Order no.
RTS Linear Template Kit Plus	20 reactions	BR1402401
RTS pIX3.0 Vector	1 vector, 25 µg	BR1402701
RTS 100 <i>E. coli</i> HY Kit	24 reactions	BR1400101
RTS 100 <i>E. coli</i> HY Kit	96 reactions	BR1400102
RTS 500 ProteoMaster <i>E. coli</i> HY Kit	5 reactions	BR1400201
RTS 9000 <i>E. coli</i> HY Kit	1 reaction	BR1400301
RTS 100 <i>E. coli</i> Disulfide Kit	24 reactions	BR1400401
RTS 500 <i>E. coli</i> Disulfide Kit	5 reactions	BR1400501
RTS pIVEX <i>E. coli</i> His-tag, 2nd Gen. Vector Set	2 vectors, 10 µg each	BR1400701
RTS Wheat Germ LinTempGenSet, His6-tag	96 reactions	BR1401201
RTS pIVEX Wheat Germ His6-tag Vector Set	2 vectors, 10 µg each	BR1401301
RTS 100 Wheat Germ Kit	24 reactions	BR1402501
RTS 100 Wheat Germ CECF Kit	24 reactions	BR1401001
RTS 500 Wheat Germ CECF Kit	5 reactions	BR1401101
RTS 500 Adapter	1 adapter	BR1401901
RTS GroE Supplement	For 5 reactions of 1 ml	BR1401701
RTS DnaK Supplement	For 5 reactions of 1 ml	BR1401601
RTS Amino Acid Sampler	1 set	BR1401801
RTS 100 Insect Membrane Kit	5 reactions	BR1401501
RTS 100 Insect Membrane Kit	20 reactions	BR1401502
RTS Linear Template Fab Kit	96 reactions	BR1402201
RTS pIX4.0 Insect Vector	1 vector, 25 µg	BR1400901