For life science research only. Not for use in diagnostic procedures. FOR *IN VITRO* USE ONLY.

Rapid Translation System RTS 100, *E.coli* HY Kit

In vitro protein synthesis system based on *E.coli* lysate

Cat. No. 3 186 148
For 24 synthesis reactions of 50 µl

Store this kit at −15°C to −25°C

Instruction Manual

Version 1, May 2001

Roche

1. Preface

1.1 Table of contents

1.2 Kit Contents

Kit contents

Each Kit contains sufficient reagents for 24 × 50 µl coupled reactions.

Vial	Label	Contents and function
1 red cap	*E.coli* Lysate	• lysate from *E.coli*, stabilized and lyophilized • contains components for transcription and translation • one bottle
2 green cap	Reaction Mix	• substrate mix to prepare reaction solution, stabilized and lyophilized • one bottle
3 brown cap	Amino Acids	• mix of 19 amino acids to prepare Reaction Solution, stabilized and lyophilized • one bottle
4 yellow cap	Methionine	• methionine to prepare Reaction Solution, stabilized and lyophilized • one bottle
5 white cap	Reconstitution Buffer	• buffer solution for the reconstitution of bottles 1, 2, 3, and 4 • one bottle containing 1.6 ml
6 colorless cap	Control Vector GFP	• 50 µg plasmid, lyophilized • GFP (green fluorescent protein) expression vector to be used for the control reaction
7	Reaction tubes	• 200 µl reaction tubes RNase and DNase free • 24 reaction tubes

Safety information

Bottles 2, 3 and 4 contain < 25% dithiothreitol. All other bottles contain no hazardous substances in reportable quantities. The usual precautions taken when handling chemicals should be observed. Used reagent can be disposed off in waste water in accordance with local regulations. In case of of eye contact flush eyes with water. In case of skin contact wash off with water. In case of ingestion seek medical advice.

Laboratory requirements

To avoid contamination with RNases we recommend using RNase-free materials and wearing gloves. Heat-treated glassware is not essential, since solutions can be reconstituted in the bottles provided.

Additional equipment required

To perform protein synthesis reactions with this kit, you will need the following equipment:

- Water bath or heater adjustable to 30°C.
  Optimal results are obtained if the reaction is stirred or shaken during incubation
- Pipettes 0–10 µl, 10–200 µl, 200–1000 µl,
- Pipette tips autoclaved at 121°C for 20 min

Additional reagents required

- Besides the template vector coding for the protein of interest, pIVEX cloning vectors or oligonucleotide primers plus PCR reagents to create a DNA expression construct are needed.
- For optional radioactive labeling [^{35}S]-methionine (> 1000 Ci/mmol at 15 mCi/ml)
- For reconstitution of the GFP Control Vector do only use deionized DNase- and RNase-free water.
- A GFP standard protein (e.g. r-GFP from Roche Molecular Biochemicals) is required for the accurate quantitation of GFP (control reaction) on SDS polyacrylamide gels.

2. Introduction

2.1 Product overview

Reaction principle

Transcription and translation take place simultaneously in a 50 µl reaction. Protein is synthesized for up to 6 hours yielding up to 20 µg of functionally active protein.

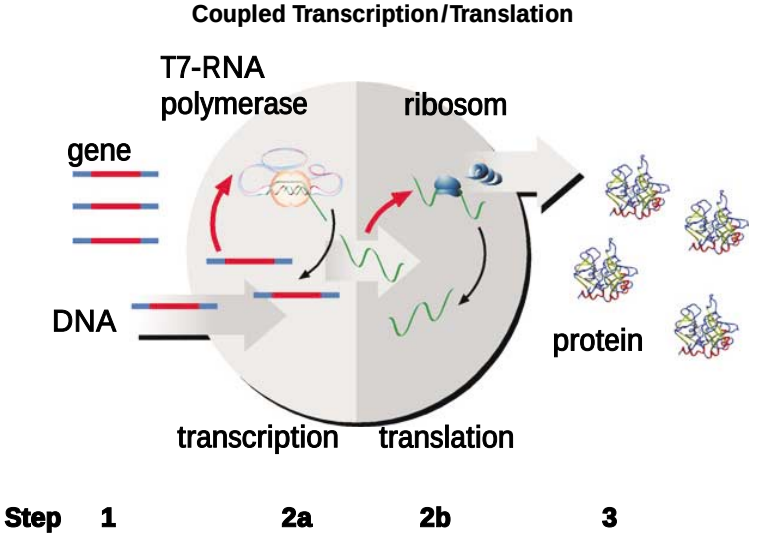

Fig. 1: Basic steps

Step	Description
1	To a gene of interest, the necessary regulatory elements like T7 promoter, ribosomal binding site and T7 terminator are added by a PCR reaction or by cloning it into a pIVEX vector.
2a and 2b	In a coupled in vitro reaction first the template DNA is transcribed into mRNA by T7 RNA polymerase, followed by translation into protein by the ribosomal machinery present in the *E.coli* lysate.
3	Expressed protein accumulates during the reaction and is harvested after a 1–6 hours run.

2.1 Product overview, continued

Application

The Rapid Translation System RTS 100 *E.coli* HY Kit is designed for

- Rapid parallel protein synthesis reactions.
- Compatiblity with both, PCR generated templates, and plasmids.
- Rapid optimization of expression constructs.
- Rapid functional testing of PCR-generated mutations
- Expression of toxic gene products.
- Synthesis of truncated gene products from PCR-generated or restriction enzyme digested DNA, for epitope or functional domain mapping.
- Expression from in vitro-synthesized RNA for genes lacking an appropriate *E.coli* promoter
- Successful synthesis of proteins in the molecular weight range from 10 to 120 kD.

Limitations of post-translational modifications

The *E.coli* lysate system can not introduce these post-translational modifications:

- Glycosylation
- Phosphorylation
- Disulfide bond formation
- Signal sequence cleavage

Template DNA

Use pIVEX plasmid or simply design PCR primers to attach T7 transcription/translation regulatory elements to your target gene to produce the template for protein expressions.

Other vectors, designed for prokaryotic in vitro protein expression and containing a T7 promoter as well as a Ribosomal Bindung Site may also be used. The requirements are described in chapter 3.2.1.

Reaction time

Between 1 and 6 hours

Number of reactions

The kit provides reagents and reaction vessels for 24 × 50 µl reactions.

Stability

This product is stable at −15°C to −25°C until the expiration date printed on the label.

Advantages

Benefits	Features
• Express your desired protein within hours	• Use pIVEX plasmid or a PCR reaction to produce the template for protein expressions. • Express 2–20 μg protein within 2–6 hours.
• Convenient	• Prepare your protein expression reaction in less than 30 minutes. • No fermentation, no safety regulations as for recombinant organisms.
• Express multiple proteins in parallel	• Perform parallel expression of both prokaryotic and eukaryotic proteins with a system that is independent of codon usage due to an excess of all tRNA species. • Produce proteins with tags or reporter genes for easy purification and detection
• Flexible	• Alter reaction conditions as needed to overcome insolubility or folding problems commonly associated with bacterial systems. • Express proteins in either microtubes or microwell plates, and adjust reaction volumes to meet your yield requirements. • Express toxic proteins that are impossible to produce in bacteial expression systems. • Incorporate radio-labeled methionine into the protein product
• Reliable quality	• Kits are tested to yield at least 400 μg/ml of functionally active GFP control protein. • Lyophylized bacterial lysate offers unprecedented stability • Good reproducibility.
• Complete	• All necessary components for the expression reactions are supplied with the kit.

2.2 Background Information

Coupled *in vitro* transcription/ translation

Instead of working with isolated mRNA it is more convenient to produce the mRNA immediately in an *in vitro* expression system (Zubay (1973)).

Upon addition of the DNA template and T7 RNA polymerase to the DNA-free *E.coli* lysate transcription and translation are closely coupled in time and space: While the T7 RNA polymerase transcribes the template gene, the ribosomes provided by the *E.coli* lysate start to translate the 5'-end of the nascent mRNA.

Lysate and kit components

The *E.coli* lysate is prepared with some modifications according to the method of Zubay (1973). An *E.coli* strain with the lowest exonuclease activity was selected and growth conditions were optimised to allow optimum protein expression from linear (PCR-generated) and plasmid templates.

3. Procedures and required materials

3.1 Protein synthesis reaction

3.1.1 Before you begin

Kit reagents

- Do not combine reagents from different kit lots.
- Reconstitute only the bottles needed for the experiment.
- For reconstitution of bottles 1 to 4 use only Reconstitution Buffer of this kit (bottle 5). For reconstitution of the plasmids (bottles 6) use sterile DNase- and RNase- free water.
- Store the reconstituted solutions from bottle 1 to 4 at −15°C to −25°C. Two freeze thaw cycles of the reconstituted solutions do not cause a significantly decreased activity.
- Reconstitution Buffer can be thawed in a 25°C water bath.
- Store the reconstituted plasmid at −15°C to −25°C
- Reconstitute the lyophilized reagents or thaw the solutions directly prior to use.
- Keep reconstituted reagents and working solutions on ice before use.

DNA template

Prepare and purify as detailed in section (3.2).

Equipment

- Use a water bath or an incubator adjusted to 30°C. Shaking of the reaction solution during the reaction is optional.
- Use only pipettes that are calibrated.
- Use RNase-free plastic and glassware.
- For precise quantification of the control reaction with GFP, apply the reaction products onto a SDS-polyacrylamide gel. A qualitative GFP estimation could be made using a UV-lamp (360 nm).

3.1.2 Reconstitution of reaction components

Solution	Bottle no. / cap	Reconstitution procedure	For use in
1	*E.coli* Lysate Bottle 1, red	Reconstitute the lyophilizate with 0.36 ml of Reconstitution Buffer (bottle 5), mix carefully by rolling or gentle shaking. Do not vortex!	• section 3.1.3 • solution 7
2	Reaction Mix Bottle 2, green	Reconstitute the lyophilizate with 0.30 ml of Reconstitution Buffer (bottle 5), mix by rolling or shaking.	• section 3.1.3 • solution 7
3	Amino Acids Bottle 3, brown	Reconstitute the lyophilizate with 0.36 ml of Reconstitution Buffer (bottle 5), mix by rolling or shaking.	• section 3.1.3 • solution 7
4	Methionine Bottle 4, yellow	Reconstitute the lyophilizate with 0.33 ml of Reconstitution Buffer (bottle 5), mix by rolling or shaking.	• section 3.1.3 • solution 7
5	Reconstitution Buffer Bottle 5, white	• 1.6 ml • Ready-to-use solution • The solution is stable at 4°C but can also be stored at −20°C	• solutions 1, 2, 3, 4
6	Control vector GFP Bottle 6, colorless	Briefly centrifuge down the content of the bottle. Reconstitute the lyophilizate with 50 µl of sterile DNase- and RNase- free water. The solution is stable at −20°C	• Section 3.1.5

Appearance of solutions

With the exception of the *E.coli* lysate all reconstituted lyophilizates should be clear solutions. The *E.coli* lysate remains cloudy.

3.1.3 Preparation of working solutions

Solution	Content	Reconstitution procedure	For use in
7	Reaction Solution	Into one of the supplied reaction tubes pipet the following components: 1. 12 µl E. coli Lysate 2. 10 µl Reaction Mix 3. 12 µl Amino Acids 4. 1 µl Methionine 5. 5 µl Reconstitution Buffer 6. 0.5 µg of the circular DNA template or 0.5 µg of linear template in 10 µl of water or TE-buffer. • A premix of solutions 1–5 without DNA is recommended for multiple parallel reactions. • Mix carefully by rolling or gentle shaking. • *DO NOT VORTEX!*	section 3.1.4

3.1.4 Running an experiment

Starting the run

- Close the reaction tubes with the supplied caps.
- Place the loaded reaction tubes into an incubator or water bath.

End of run

- After 4–6 hours remove the reaction tube from the incubator.
- Store reaction solution frozen or at 0–4°C until purification or further processing.

Points to consider

Parameter	Guideline
Temperature	Optimal temperature for most proteins is 30°C. However, lower temperatures may be used for proteins that tend to aggregate.
Time	Protein synthesis continues for up to 6 hours, about 90% of the reaction is finished, however, after 4 hours.

3.1.5 GFP control reaction

Reaction procedure

Step	Action
1	Reconstitute bottles 1 to 4 and bottle 6 according to chapter 3.1.2.
2	Prepare Reaction Solution in one of the reaction tubes according to chapter 3.1.2 and 3.1.3. Add 0.5 µg (0.5 µl) of reconstituted Control Vector GFP (bottle 6).
3	Start the reaction: Temperature: 30°C Time: 6 hours
4	After the run remove Reaction Solution and store in a 2 ml vial for 24 hours at 4°C for maturation of GFP.
5	The fluorescence of GFP (excitation wavelength 395 nm, emission wavelength 504 nm) can be observed using an UV lamp (360 nm)
6	Apply 2–5 µl of the reaction onto SDS-polyacrylamide gels and add 1 µl of a GFP standard (separately available) onto a separate lane.
7	Run the gel, then stain with Coomassie Blue and quantify the synthesized GFP by comparision with the GFP standard. ***Note:*** The GFP protein can also be detected on Western blots by using an anti-His antibody.

Oxidation of GFP

GFP needs molecular oxygen to form the fluorophor post-translationally (Coxon, A. & Bestor, T. H. (1995)). The yield of properly-folded fluorescent GFP is further increased by storing the Reaction Solution after the expression for 24 hours at 4°C.

After 24 hours of storing at 4°C the maturation is almost completed.

3.1.6 Radioactive labeling

Reaction procedure

Step	Action
1	Reconstitute bottles 1 to 4 and bottle 6 according to chapter 3.1.2.
2	Dilute 10 µl of the reconstituted Methionine solution of 3.1.2 with 990 µl of nuclease-free water to yield a 1 mM Methionine solution
3	For one radioactive reaction prepare the following Reaction Solution in one of the reaction tubes: 1. 6 µl E. coli Lysate 2. 5 µl Reaction Mix 3. 6 µl Amino Acids 4. 1.25 µl 1 mM Methionine solution (see Note) 5. 2 µl of a L-[^{35}S]Methionine (SJ 235 Amersham) 15 mCi/ml 6. 2.5 µl Reconstitution Buffer 7. 0.25 µg of the plasmid DNA or 0.25 µg of linear template in 2.25 µl of water or TE-buffer. ***Note***: Addition of unlabeled methionine to the labeling reaction is required to prevent premature termination for larger proteins or proteins with many methionine residues.
4	Start the reaction Temperature: 30°C
5	Time: Take 5 µl samples after 20, 30 and 60 min
6	Apply 2–5 µl of the reaction samples onto SDS-polyacrylamide gels.
7	After the separation, dry the gel and apply it onto Kodak X-OMAT AR films for autoradiography (3 hrs exposition time).

3.2 Preparation of DNA templates

Literature

Basic molecular biology methods may be found in one of the following method books:

- Sambrook et al (1989) “Molecular Cloning - A Laboratory Manual” Second Edition, Cold Spring Harbor Laboratory Press, New York.
- Ausubel, U. K. et al (1993) “Current Protocols In Molecular Biology” John Wiley & Sons Inc., New York.

3.2.1 Use of plasmid vectors or restriction fragments of plasmid vectors

Necessary vector elements

Any vector or vector fragment to be used in combination with the Rapid Translation System must include the following elements and structural features:

- target gene under control of T7 promoter located downstream of a RBS (ribosomal binding site) sequence
- distance between T7 promoter and start ATG should not exceed 100 base pairs
- distance between the RBS sequence and start ATG should not be more than 5–8 base pairs
- T7 terminator sequence at the 3’ end of the gene

General recommendations

The pIVEX vector family has been developed and optimized for use in the Rapid Translation System. Therefore, we strongly recommend cloning target genes into a pIVEX vector prior to expression.
A schematic view of some of the available pIVEX vectors is given in Fig 3. Whole vector sequences and vector maps could be loaded from the Roche web page (www.proteinexpression.com).

Purity of the plasmid preparation

The purity of plasmids obtained from commercially available DNA preparation kits is sufficient for the use as template in the Rapid Translation System. When DNA purity is unsufficient ($OD_{260/280} \leq 1.7$), a phenol extraction to remove traces of RNase may enhance expression.

Note: Do not purify DNA-fragments over agarose gels, as this treatment inhibits *in vitro* protein synthesis.

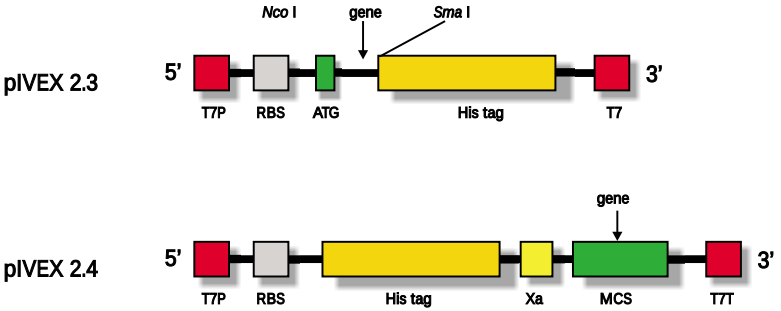

Fig. 2: Functional elements of cloning vectors

Abbreviations:

T7 P	= T7 Promotor
RBS	= Ribosomal binding site
ATG	= Start codon
Nco I, Sma I	= restriction enzyme recognition sites for cloning of the target gene
C-Tag, N-Tag	= C- or N-terminal tag position
Xa	= Factor Xa restriction protease cleavage site
MCS	= Multiple cloning site in three different reading frames for the insertion of the target gene
T7 T	= T7 Terminator

3.2.2 Generation of Expression Templates by PCR

Principle applications

The use of PCR protocols instead of subcloning allows rapid expression of new or modified DNA-templates:

- Addition of a strong T7 promoter to an existing expression plasmid
- Addition of Tag sequences for detection with antibodies
- Introduction of mutations
- Change of codon usage
- Truncation of proteins

Introduction of a T7 promotor via a 1-step PCR protocol

Step	Action
1	• Design a primer that contains the T7 promotor sequence and a 15–20 nucleotide sequence homologous to the plasmid already containing a prokaryotic ribosomal binding site and the gene of interest. • Design a primer that contains the T7 terminator sequence and a 15–20 nucleotide sequence homologous to the plasmid and located 200–300 basepairs downstream in 3´-direction to the gene of interest. (*For an example see section 4*)
2	Perform a PCR reaction using these primers.
3	Determine the concentration of the PCR product by an agarose gel and comparision to known amounts DNA molecular size markers.
4	Use the PCR product directly for *in vitro* protein synthesis, or after purification with the PCR Clean Up Kit, which can be used to remove potentially inhibitory primer dimers. ***Note:*** Do not purify DNA-fragments over agarose gels, as this treatment inhibits *in vitro* protein synthesis.

Addition of a C-terminal Tag sequence for detection by a 1-step PCR protocol

Step	Action
1	• Design a primer that is located upstream of a T7 promotor sequence in a prokaryotic expression plasmid with the gene of interest and the necessary elements or that contains a T7 promoter sequence and the regulatory elements as detailed in section 3.2.1. • Design a primer that contains the T7 terminator sequence, a spacer region of at least 25 nucleotides distance to the stop codon followed by the tag sequence and a 15–20 basepair sequence homologous to the gene of interest. (*For an example see section 4*)
2–4	Perform a PCR reaction using these primers and follow steps 3 and 4 as in the protocol above.

4. Typical results

Kit performance

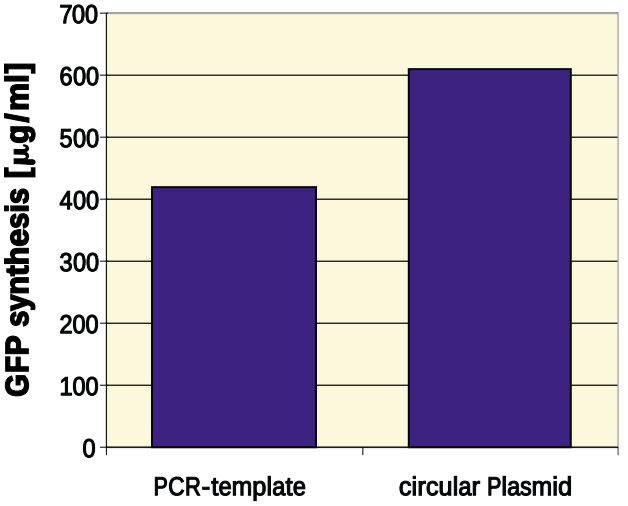

Fig. 3: A GFP coding DNA template generated by 1-step PCR or cloning into the pIVEX2.3 expression plasmid was used for the *in vitro* protein synthesis reactions. Up to 400 µg/ml of protein from linear and up to 600 µg/ml from plasmid template were obtained in a of 4 hour reaction.

Addition of a C-terminal Tag sequence

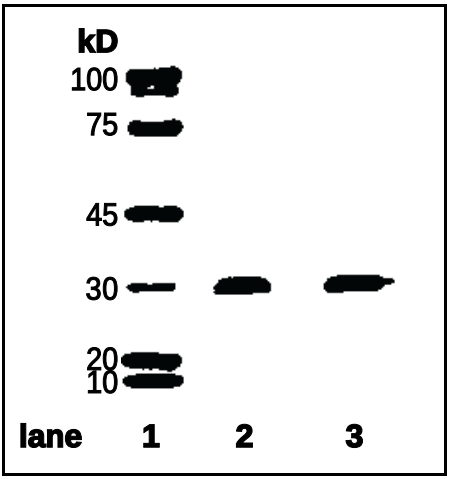

Fig. 4: Western Blot with anti-His_6 antibody
Lane 1: GFP-His; PCR product from pIVEX2.3-GFP
Lane 2: GFP-Strep; PCR product from pIVEX-GFP without Histag
Lane 3: GFP with His introduced via primer; PCR product from pIVEX -GFP

Starting with a pIVEX vector containing a GFP gene without a tag as a template a 1-step PCR with the following primers was performed to introduce a C-terminal hexa-his Tag
Sense primer
5' GAAATTAATACGACTCACTATAGGGAGACCACAACGGTTTC 3´
Antisense primer
5´-CAAAAAACCCCTCAAGACCCGTTTAGAGGCCCCAAGGGGTTGG GAGTAGAATGTTAAGGATTAGTTTAT-TAATGATGATGATGATGATGTTTGTATAGTTCATC

Introduction of a T7 promotor via a 1-step PCR protocol

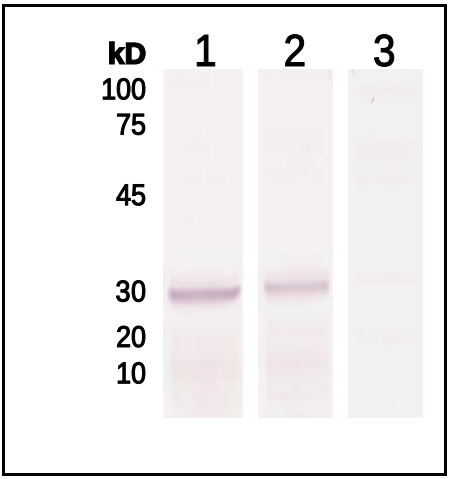

Fig. 5: Western Blot with anti-His_6 antibody
Lane 1: His-Phosphatase; PCR product with sense primer 1 from pQE
Lane 2: His-Phosphatase; PCR product with sense primer 2 from pQE
Lane 3: His-Phosphatase; PCR product without T7 Primer from pQE

Starting with a pQE vector containing a phosphatase downstream of an *E.coli* promotor, a 1-step PCR with two different sense primers was performed to introduce a T7 Promotor

Sense primer 1
T7 Promotor (in italics); Underlined sequences: overlap region between primer and vector.
5'-GAAAT*TAATA CGACTCACTATA*GGGAGACC ACAACGGTTT CCCTAACAAT TATAATAGAT TCA-3´
Sense primer 2
5´-GAAAT*TAATACGACTCACTATA*GGGAGACCACAACGGTTTCCCTC TAGAATAATTTTGTTTAACTTTAAGAAGGAGAAATTAACTATG -3´
Antisense primer:
T7 Terminator (in italics); Underlined sequences: overlap region between primer and vector.
5´-*CAAAAAACCCCTCAAGACCCGTTTAGAGGCCCCAAGGGGG* GCCAAAGGCCGGATAAAACTTGTGCT -3´

Radioactive labeling with L-[^{35}S]Methionine

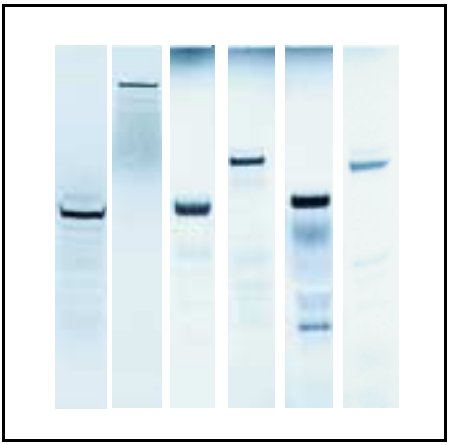

Fig 6: Autoradiography of *in vitro* synthesized protein labeled with L-[^{35}S]Methionine
Lane 1: GFP
Lane 2: β-galactosidase
Lane 3: Fc gamma protein
Lane 4: Rec. Plasminogen activator
Lane 5: Single chain antibody fragment
Lane 6: Rhodanese
The indicated genes were cloned into pIVEX2.3 vectors and amplified by a 1-step PCR with 5' GAA ATT AAT ACG ACT CAC TAT AGG GAG ACC ACA ACG GTT TC 3´ as sense primer and 5' CAA AAA ACC CCT CAA GAC CCG TTT AGA GGC CCC AAG G 3´ as antisense primer. The PCR generated template was used for *in vitro* expression without further purification.

5. Appendix

5.1 Trouble shooting

5.1.1 General problems

Problem	Possible Cause	Recommendation
No expression during the control reaction.	Kit expired.	Order a new kit.
	Kit has not been stored at −20°C.	Order a new kit.
	Contamination with RNases.	Repeat experiment and take care to work RNase-free at each step.
	A kit component may be bad.	Contact Roche Diagnostics.

5.1.2 Problems with the expression of the target protein, while the GFP control reaction works

Problem	Possible Cause	Recommendation
Good protein expression, but low yield of active protein.	Incorrect folding of the protein due to: Dependence on cofactors • Necessity of disulfide bond formation. • Dependence on secondary modifications • Dependence on chaperones	• Add necessary cofactors. • Oxidize after the reaction to form disulfide bonds (see e.g. Ahmed et al (1975), Odorzinsky & Light (1979)) • The *E.coli* lysate can not introduce post-translational modifications like glycosylation, phosphorylation, or signal sequence cleavage. • Add chaperones (see e.g. Rudolph (1997)
Product in the pellet fraction.	Aggregation	• Add / adjust chaperones. • Adjust experimental conditions (time, temperature). • Add mild detergents (e.g. up to 0.1% Triton X-100 (v/v) or 0.1% Chaps (w/v) for membrane proteins).

continued on next page

5.1 Trouble shooting, continued

Problem	Possible Cause	Recommendation
Low expression yield.	Expression time too short.	Extend expression time.
	The tag has a negative influence on the folding of the protein.	Try different pIVEX vectors. Introduce different Tag sequences via PCR.
	Amount of template DNA not optimal	Vary the DNA concentration for a 50 µl reaction between 0.1 µg to 1 µg for circular templates and linear templates to get optimum results.
Several product bands on SDS-PAGE or product smaller than expected.	Proteolytic degradation.	• Add protease inhibitors to the reaction. Dissolve 1 tablet of Complete mini EDTA-free in 0.5 ml nuclease- free water and add 2 µl for a 50 µl reaction. Use up to 10 mM EGTA.
	Internal initiation site	Replace the corresponding methionine by point mutation.
	Premature termination of the translation.	• Check the sequence of the target gene: reading frame, mutation yielding a stop codon? • Search for strong secondary structures of the mRNA and eliminate them by conservative mutations. • Increase the amount of unlabeled metrhionine during radioactive labeling, or decrease the reaction time.
No expression of the target gene, but normal expression of GFP.	Cloning error.	Check the sequence.
	Low purity of template DNA	• Assure that 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.
	Contamination with RNases.	Repeat experiment and take care to work RNase-free at each step.
	No initiation of translation due to strong secondary structures of the mRNA	• Try expression in different pIVEX-vectors. • Try expression as N-terminally tagged fusion protein in pIVEX 2.4.
	Expressed protein interferes with the translation or transcription process.	• Express the gene of interest together with GFP. If GFP expression is inhibited the active protein can not expressed with the kit.

5.2 References

1 Zubay, G (1973) Annu. Rev. Genet. **7**, 267.

2 Spirin, A. S. et al (1988) "A continuous cell-free translation systemcapable of producing polypeptides in high yield" Science **242,** 1162.

3 Coxon, A. & Bestor, T. H. (1995) Chemistry & Biology **2,** 119.

4 Sambrook et al (1989) "Molecular Cloning - A Laboratory Manual" Second Edition, Cold Spring Harbor Laboratory Press, New York.

5 Ausubel, U. K. et al (1993) "Current Protocols In Molecular Biology" John Wiley & Sons Inc., New York.

6 Ahmed, A. K. et al (1975), J. Biol. Chem. **250**, 8477.

7 Odorzinsky, T. W. & Light, A. (1979), J. Biol. Chem. **254**, 4291.

8 Rudolph, R. et al (1997) in "Protein Function – A Practical Approach" Creighton, T. E. ed. Oxford University Press Inc. New York, pp 57 - 99.

5.3 Notice for the Purchaser

5.4 How to contact Roche Molecular Biochemicals

Three ways to contact us

To contact Roche Molecular Biochemicals for technical assistance, please choose one of the following

IF you are using...	THEN...
the Internet	type our web-site address: http://biochem.roche.com
E-mail	please refer to the address which corresponds to your particular location, printed at the end of this package insert
telephone	please refer to the telephone number which corresponds to your particular location, printed at the end of this package insert

5.5 Related products

Product	Pack size	Cat. No.
RTS 500 Instrument	1 piece	3 032 060
Rapid Translation System RTS 500 *E. coli* Circular Template Kit	1 Kit, 5 reactions	3 018 008
r-GFP	50 µg	1 814 524
Rapid Translation System RTS pIVEX His-tag Vector Set	1 Set of 5 vectors, 10 µg each	3 253 538
Expand High Fidelity PCR-System	10 × 250 units	1 759 078
Agarose MP	500 g	1 388 991
Anti-His6-Peroxidase	50 U	1 965 085
PCR Clean Up Kit	1 Kit	1 696 513
Complete Mini, EDTA-free	25 tablets	1 836 170

6. Quick Reference Procedure

Reconstitution of reaction components and run of a reaction

Step	Bottle no. / cap color	Reconstitution
1	*E.coli* Lysate Bottle 1, red	Reconstitute with 0.36 ml of Reconstitution Buffer DO NOT VORTEX!
2	Reaction Mix Bottle 2, green	Reconstitute with 0.30 ml of Reconstitution Buffer.
3	Amino Acids Bottle 3, brown	Reconstitute with 0.36 ml of Reconstitution Buffer.
4	Methionine Bottle 4, yellow	Reconstitute with 0.33 ml of Reconstitution Buffer.
5	Control vector GFP Bottle 6, colorless	Briefly centrifuge down the contents of the bottle. Reconstitute the lyophilizate with 50 µl of sterile DNase- and RNase- free water.
6	Reaction Solution	Mix the following components: 1. 12 µl *E.coli* Lysate 2. 10 µl Reaction Mix 3. 12 µl Amino Acids 4. 1 µl Methionine 5. 5 µl Reconstitution Buffer 6. 0.5 µg of the circular DNA template or 0.5 µg of linear template in 10 µl of water or TE-buffer.
7	Start of the reaction	Incubate for 4–6 hours at 30°C

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biochem.cn@roche.com	China
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