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# Rapid Translation System RTS Amino Acid Sampler

Amino acid solutions for use in RTS 500 cell-free protein expression

**Cat. No. 3 262 154** Store the kit at −15 to −25°C

For 5 RTS 500 reactions

## *Instruction Manual*

Version 1, August 2001

☞ **The Rapid Translation System RTS website:**
**www.proteinexpression.com**

Roche

# 1. Preface

## 1.1 Table of contents

## 1.2 Kit Contents

| Vial | Label | Contents and function |
|---|---|---|
| 1 | Alanine<br>Ala, A | One vial each, containing 1.0 ml 42 mM buffered L-amino acid solution. |
| 2 | Arginine<br>Arg, R | |
| 3 | Asparagine<br>Asn, N | |
| 4 | Aspartic Acid<br>Asp, D | |
| 5 | Cysteine<br>Cys, C | |
| 6 | Glutamine<br>Gln, Q | |
| 7 | Glutamic Acid<br>Glu, E | |
| 8 | Glycine<br>Gly, G | |
| 9 | Histidine<br>His, H | |
| 10 | Isoleucine<br>Ile, I | |
| 11 | Leucine<br>Leu, L | |
| 12 | Lysine<br>Lys, K | |
| 13 | Methionine<br>Met, M | |
| 14 | Phenylalanine<br>Phe, F | |
| 15 | Proline<br>Pro, P | |
| 16 | Serine<br>Ser, S | |
| 17 | Threonine<br>Thr, T | |
| 18 | Tryptophan<br>Trp, W | |
| 19 | Tyrosine<br>Tyr, Y | |
| 20 | Valine<br>Val, V | |
| 21 | DTT<br>1,4-Dithiothreitol | Five vials, each containing 0.5 ml 40 mM DTT |

**Safety Information**

None of the bottles contains 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 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.

**Additional reagents required**

- The Rapid Translation System RTS Amino Acid Sampler is designed for use with the Rapid Translation System RTS 500 *E. coli* HY Kit (Cat. No. 3 246 817; 2 reactions and 3246 949; 5 reactions).
- Modified amino acids for combination with the amino acids provided in the sampler must be obtained separately.

## 2. Introduction

### 2.1 Product overview

**System components and application**

The Rapid Translation System RTS 500 consists of the RTS 500 Instrument and the RTS 500 *E.coli* HY Kit which contains reagents and reaction devices for 2 (Cat. No. 3 246 817) or 5 (Cat. No. 3 246 949) coupled transcription/translation reactions.

The amino acids in the RTS 500 *E.coli* HY kit are provided in a separate tube apart from other reaction components and can therefore be exchanged for customized amino acid mixtures, e.g. for production of labeled proteins for NMR-spectroscopy. The RTS Amino Acid Sampler provides the basis for preparing these customized mixes, in which single amino acids are exchanged. By providing each amino acid separately, any mixture can be prepared.

**Stability**

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

All components have been tested for their stability after freezing and thawing. No decrease in expression level was observed after 5 freeze-thaw cycles.

DTT (Vial 21, five vials) should be used only once after opening a vial. A separate vial is used for each RTS 500 reaction.

## 2.2 Background Information

**Coupled *in vitro* transcription/ translation using the Rapid Translation System**

NMR spectroscopy plays an increasingly important role in structural biology since it not only allows to solve the 3D-structure of the protein of interest but also to investigate dynamics and intermolecular interactions (1, 2, 3).

Cell-free protein synthesis has been shown to have exceptional advantages when proteins have to be labeled for NMR:

- the protein of interest is expressed from an exogenous plasmid template and is the only protein produced and labeled,
- the translation reaction is performed in a small volume, requiring relatively low amounts of labeled amino acids
- the metabolism of amino acids in a cell free system is lower compared to cellular systems, leading to more amino acid-specific labeling
- no expensive media are required since no cells need to be cultivated

Using the RTS 500 *E.coli* HY Kit in combination with the RTS Amino Acid Sampler and selected labeled amino acid(s), it is possible to label any amino acid of choice in the protein of interest. The complexity of NMR-spectra can be greatly reduced by amino acid-specific labeling (2, 3).

**Testing protein expression in the Rapid Translation System**

The RTS 500 *E.coli* HY Kit is based on an *E.coli* lysate and has restrictions for the expression of certain classes of proteins. To test whether a protein is successfully expressed in RTS, we recommend to start with a small-scale test expression in the RTS 100 *E.coli* HY Kit (Cat. No. 3 186 148). This kit allows the fast and economic production of protein in a 50 µl reaction.

After positive reactions we recommend to proceed with the expression on a larger scale using the RTS 500 *E.coli* HY Kit.

For a detailed description of the Rapid Translation System please refer to the pack insert of the RTS 500 *E.coli* HY Kit or visit www.proteinexpression.com

## 3. Procedures and required materials

### 3.1 Before you begin

**Reagents**

- First prepare the amino acid mixture (section 3.2), then reconstitute the required reaction components of the RTS 500 *E. coli* HY Kit (Section 3.3).
- The contents of the RTS Amino Acid Sampler can be thawed at room temperature. Some amino acids show some precipitation after thawing, which disappears after warming to 37–42°C.
- Reconstitute only the bottles from the RTS 500 *E.coli* HY Kit needed for the experiment, using only the reconstitution buffer provided in the RTS 500 *E.coli* HY Kit.
- Keep reconstituted reagents and working solutions on ice before use.

**Equipment**

- Use the Rapid Translation System RTS 500 Instrument from Roche Molecular Biochemicals for optimal reaction performance and convenience (Cat. No. 3 064 859)
- Use only calibrated pipettes.
- Use RNase-free plastic and glassware.

## 3.2 Preparation of Amino Acid Mixture

**Preparation of labeled amino acid stock solution**

A 42 mM stock solution of a labeled amino acid is recommended for labeling during RTS 500 *E.coli* HY protein expression. The amino acid solutions provided in the sampler are all adjusted to 42 mM, leading to a final concentration of 2.1 mM for each amino acid in the amino acid mix and 0.5 mM for each amino acid in the final RTS 500 reaction.

For adjustment of concentration the use of the Reconstitution Buffer (Bottle 6) from the RTS 500 E. coli HY Kit is recommended.

Some amino acids require additional treatment for dissolving and stability. When dissolving

- Tryptophan, adjust pH to 7.1 with HCl 0.1 M (approx. 50 µl required for 1 ml Trp),
- Tyrosine, adjust pH to 10.0 with KOH 2M (approx. 165 µl required for 1 ml Tyr),
- Cysteine, add DTT to a final concentration of 8 mM,
- Methionine, add DTT to a final concentration of 4 mM.

**Preparation of amino acid labeling mix**

The amino acids in the RTS 500 Amino Acid Sampler are arranged in a grid (see Fig. 1), supporting the error-free combination of 20 amino acids to a functional mix.

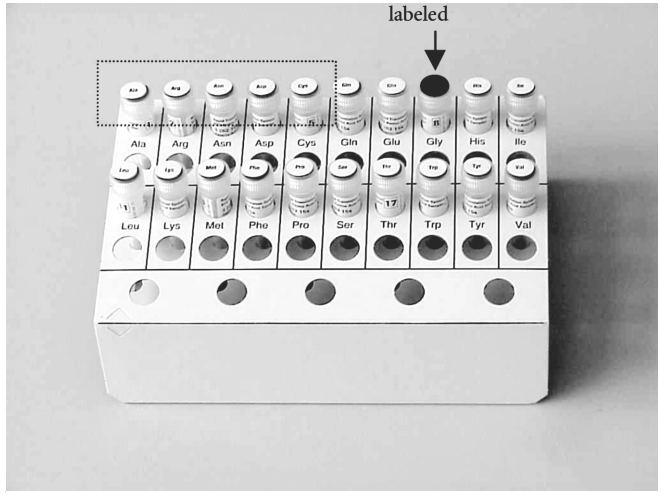



**Fig. 1: Before preparing the mix all amino acids are in their basic position.**

We recommend the removal of the amino acid(s) to be labeled from the grid and the replacement by the labeled amino acid stock solution(s) on the open position(s) (as indicated by a black capped tube for Gly in Fig. 1 and 2). The mix can now be prepared by pipetting 150 µl per amino acid, using a fresh pipette tip for each amino acid. Use an RNase free tube (e.g. 15 ml Falcon) for preparation of the mix. The final volume of the mix is 3.0 ml.

By moving the vial to the next row after pipetting there is a visual check for which amino acids already have been combined. The risk of leaving amino acids out or double pipetting of amino acids is thus minimized.

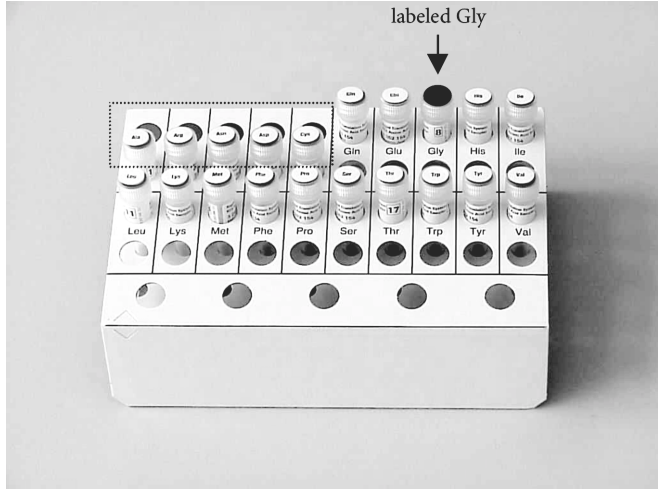



**Fig. 2: After being pipetted from its tube, each amino acid is moved to the next row (result after 5 amino acids shown boxed). The progress of mixing can easily be monitored this way.**
After pipetting all 20 amino acids the solution is mixed thoroughly by vortexing. The amino acid labeling mix is now ready for addition to reaction and feeding solution (See Section 3.3.3.).

## 3.3 Preparation of reactions components

### 3.3.1 Reconstitution of reaction components from the RTS 500 *E.coli* HY kit

**Using the RTS 500 *E.coli* HY Kit**

The Rapid Translation Kit RTS 500 *E.coli* HY Kit is available in 2 pack sizes:
Cat. No. 3 246 817 for two 1 ml reactions
Cat. No. 3 246 949 for five 1 ml reactions

When combined with the RTS Amino Acid Sampler, the Amino Acid Mix without Methionine (Bottle 4, brown cap) and Methionine (Bottle 5, yellow cap) contained in the Rapid Translation Kit RTS 500 *E.coli* HY Kit are not required.

| Component | Reconstitution/Preparation of working solution | For use in |
|---|---|---|
| *E. coli* Lysate<br><br>Bottle 1, red cap | Reconstitute the lyophilizate with 0.525 ml of Reconstitution Buffer (bottle 6). Mix gently.<br>DO NOT VORTEX! | • Section 3.3.3<br>• Reaction Solution |
| Reaction Mix<br><br>Bottle 2, green cap | Reconstitute the lyophilizate with 0.25 ml of Reconstitution Buffer (bottle 6), mix by rolling or shaking. | • Section 3.3.3<br>• Reaction Solution |
| Feeding Mix<br><br>Bottle 3, blue cap | Reconstitute the lyophilizate with 8.1 ml of Reconstitution Buffer (bottle 6), mix by rolling or shaking. | • Section 3.3.3<br>• Feeding Solution |
| Reconstitution Buffer<br><br>Bottle 6, white cap | • Ready-to-use solution<br>• The solution is stable at 4°C but can also be stored at −20°C | |

### 3.3.2 Reaction Components from the RTS Amino Acid Sampler

| Component | Reconstitution/Preparation of working solution | For use in |
|---|---|---|
| Amino acids labeling mix<br><br>Vial 1-20 | Prepare a 2.1 mM stock solution of all amino acids in 3 ml of Reconstitution Buffer as described under Section 3.2. | Section 3.3.3. Reaction Solution and Feeding Solution |
| DTT<br><br>Vial 21 | Ready-to-use solution<br>Open each vial only once<br>Repeated freezing and thawing is allowed | Section 3.3.3. Reaction Solution and Feeding Solution |

### 3.3.3 Preparation of working solutions

| Content | Reconstitution/Preparation of working solution |
|---|---|
| Feeding Solution | To the content of bottle 3 (Feeding Mix) add 2.65 ml of the amino acids labeling mix (see section 3.2.) and 0.3 ml of DTT-solution (Vial 21). Mix by rolling or shaking. |
| Reaction Solution | To the content of bottle 1 (*E.coli* Lysate) add 0.225 ml of the reconstituted Reaction Mix (bottle 2), 0.27 ml of the amino acid labeling mix (see Section 3.2.) and 30 µl of DTT solution (Vial 21). Add 10–15 µg of DNA template in a maximum volume of 50 µl. Mix carefully by rolling or gentle shaking.<br>Do not vortex! |

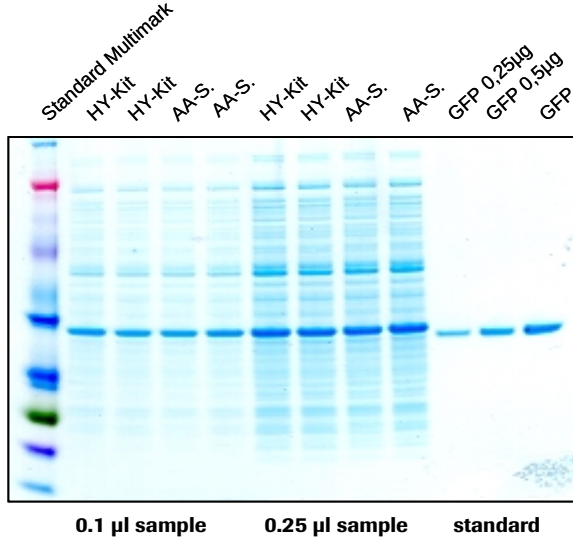
### 3.3.4 Running an experiment

**Running the experiment**

After preparation of the working solutions proceed with the procedure for loading the RTS 500 device as described in the pack insert of the RTS 500 *E.coli* 500 HY Kit under “Running an experiment”.

## 4. Typical results

Using a mixture of amino acids from the RTS Amino Acid Sampler, the protein synthesis rate was compared to the synthesis rate obtained with the Amino Acid Mix without Methionine (bottle 4) and Methionine (bottle 5) from the RTS 500 *E.coli* HY Kit. The amount of protein produced in the 1 ml standard reaction is similar (see Fig. 3)



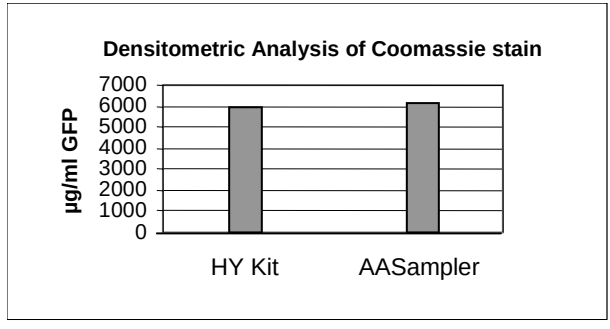




**Fig. 3:** Comparision of RTS reactions performed with the ready-to-use amino acid mixture contained in the RTS 500 *E.coli* HY kit or with amino acids provided separately in the Amino Acid Sampler. After running the RTS reaction, various amounts of sample were loaded in duplicates on a Coomassie-stained SDS-Gel. A GFP standard was loaded in parallel for subsequent densitometric analysis.

*continued on next page*

## 4. Typical results, continued

To test the incorporation of a labeled amino acid, an amino acid mixture containing $^{15}$N-Glycine (Isotech, Miamisburg, OH) was prepared according to the procedures described in this pack insert. The final concentration of labeled amino acid in the RTS reaction device Solution was 0.5mM. After synthesis the produced protein was analyzed on SDS-PAGE (See Fig. 4), showing the production of protein of expected length.

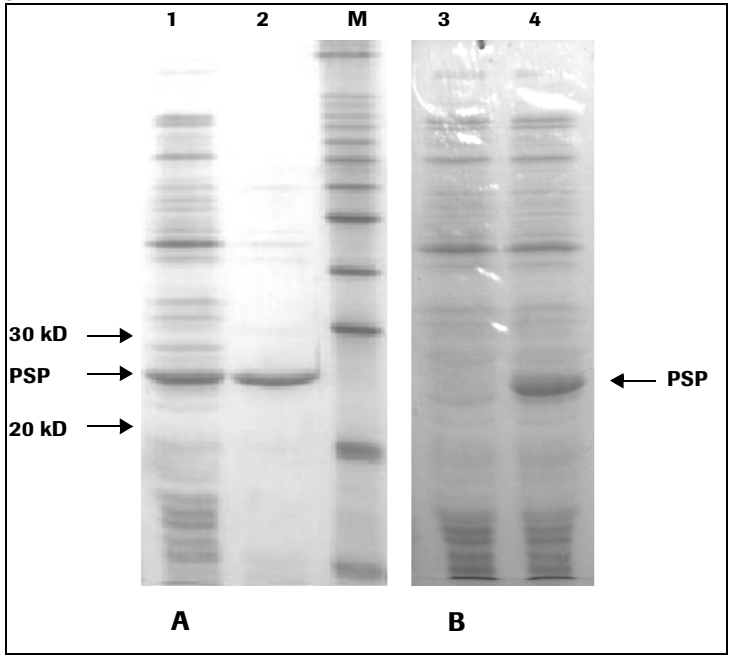



**Fig. 4:** Expression of phosphoserine phosphatase (PSP) in RTS (A) Unlabeled protein from a total RTS 500 *E.coli* HY reaction mix (lane 1) and after purification (lane 2). M: 10 kD molecular weight marker. (B) $^{15}$N-Glycine labeled protein in a total reaction mix (lane 4) compared to a reaction without expression template (lane 3) Note: this experiment was performed with a slightly modified protocol and a with a prototype of the amino acid sampler .

## 5. Appendix

### 5.1 Trouble shooting

| Problem | Possible Cause | Recommendation |
|---|---|---|
| No protein produced | The protein is difficult to express in the Rapid Translation System | Test expression first in the RTS 100 *E.coli* HY Kit. Follow the recommendations for increasing success rate provided with the kit |
| No protein produced although RTS 100 protein production was positive | The amino acid labeling mix is missing one or more amino acids | Repeat the experiment using freshly prepared mixtures. Compare expression with the expression using the amino acids provided in the RTS 500 *E.coli* HY Kit. |
| Some amino acids show precipitation after thawing | | Warm the tubes to 37–42°C. Precipitates must dissolve. |

### 5.2 References


1 Riek, R., Pervushin, K., Wuethrich, K. (2000). TROSY and CRINEPT: NMR with large molecular and supramolecular structures in solution *TIBS* **25** (2000), p. 462.

2 Gardner, K.H., Kay, L. E. (1998): The use of H, C, N multidimensional NMR to study the structure and dynamics of proteins. *Annu Rev Biophys Biomol Struct* **27** (1998), p. 357.

3 Kigawa T, Yabuki T, Yoshida Y, Tsutsui M, Ito Y, Shibata T, and Yokoyama S (1999): Cell-free production and stable isotope labeling of milligram quantities of proteins. *FEBS Lett* **442** (1999), pp.15-19.

## 5.3 How to contact Roche Molecular Biochemicals

**Ways to contact us** To contact Roche Molecular Biochemicals for technical assistance, please choose one of the following:

| IF you are using... | THEN... |
|---|---|
| Internet | access our web-site at: http:\\www.proteinexpression.com, or http:\\biochem.roche.com. |
| E-mail | Please refer to the address that corresponds to your particular location, on the last page of this instruction manual. |
| Telephone | Please refer to the address that corresponds to your particular location, on the last page of this instruction manual. |

## 5.4 Related products

| Product | Pack Size | Cat. No. |
|---|---|---|
| Rapid Translation System<br>RTS 500 Instrument | 1 instrument | 3064 859 |
| Rapid Translation System<br>RTS 100 *E.coli* HY Kit | 24 reactions (50 µl each) | 3186 148 |
| Rapid Translation System<br>RTS 500 *E.coli* HY Kit | 2 reactions<br>5 reactions | 3246 817<br>3246 949 |
| Rapid Translation System<br>RTS pIVEX His-tag Vector Set | 5 vectors (10 µg each) | 3253 538 |
| Rapid Translation System<br>RTS GroE Supplement | 5 × 125 µl | 3263 690 |
| Restriction Protease Factor Xa | 3 × 30 µg<br>3 x 100 µg<br>3 x 250 µg | 1179 888<br>1585 924<br>1179 896 |
| Restriction Protease Factor Xa Cleavage and Removal Kit | Kit I : 3 × 30 µg<br>Kit II: 3 × 100 µg | 1644 777<br>1644 785 |
| rGFP | 50 µg | 1814 524 |
| Anti-$His_6$ | 100 µg | 1922 416 |
| Anti-$His_6$-Peroxidase | 50 U | 1965 085 |
| Complete Protease Inhibitor Cocktail Tablets, mini, EDTA-free | 25 tablets (each tablet for 10 ml extract) | 1836 170 |

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