RTS™ Amino Acid Sampler Manual

For preparation of customized amino acid mixtures for use in RTS *E. coli* expression kits

RTS Amino Acid Sampler Manual, 21.03.2025

Contents

Product specifications

The RTS Amino Acid Sampler provides the basis for the expression of labeled proteins using RTS 100, 500 ProteoMaster, or 9000 *E. coli* HY expression kits. By providing each amino acid as a separate stock solution, the sampler allows the preparation of customized amino acid mixtures in which single amino acids can be exchanged with labeled ones. Using the prepared mixture in RTS *E. coli* HY expression reactions, proteins can be specifically labeled for structural studies.

Due to the high expression yields required for structural studies, the RTS Amino Acid Sampler is highly recommended for use in combination with the RTS 500 ProteoMaster *E. coli* HY Kit, or the RTS 9000 *E. coli* HY Kit. However, it may also be used with the RTS 100 *E. coli* HY Kit.

Note: Labeled amino acids are not supplied with the RTS Amino Acid Sampler and must be obtained separately.

Product description

The RTS Amino Acid Sampler contains all 20 L-amino acids as single stock solutions (20 × 1.5 ml), and 5 vials of DTT (0.8 ml).

One RTS Amino Acid Sampler contains sufficient amino acids to prepare a customized amino acid mixture for:

- Over 2,000 RTS 100 *E. coli* HY reactions
- 10 RTS 500 ProteoMaster *E. coli* HY reactions
- One RTS 9000 *E. coli* HY reaction

For detailed information about the RTS 100, 500 ProteoMaster, and 9000 *E. coli* HY expression kits, visit www.biotechrabbit.com.

Product limitations

The RTS Amino Acid Sampler 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

Vial	Label	Contents and function
	Ordering number	BR1401801
1	Alanine (Ala, A); AS Sampler	1 vial (1.5 ml; 168 mM; buffered L-amino acid solution)
2	Arginine (Arg, R); AS Sampler	1 vial (1.5 ml; 168 mM; buffered L-amino acid solution)
3	Asparagine (Asn, N); AS Sampler	1 vial (1.5 ml; 168 mM; buffered L-amino acid solution)
4	Aspartic Acid (Asp, D); AS Sampler	1 vial (1.5 ml; 168 mM; buffered L-amino acid solution)
5	Cysteine (Cys, C); AS Sampler	1 vial (1.5 ml; 168 mM; buffered L-amino acid solution)
6	Glutamine (Gln, Q); AS Sampler	1 vial (1.5 ml; 168 mM; buffered L-amino acid solution)
7	Glutamic Acid (Glu, E); AS Sampler	1 vial (1.5 ml; 168 mM; buffered L-amino acid solution)
8	Glycine (Gly, G); AS Sampler	1 vial (1.5 ml; 168 mM; buffered L-amino acid solution)
9	Histidine (His, H); AS Sampler	1 vial (1.5 ml; 168 mM; buffered L-amino acid solution)
10	Isoleucine (Ile, I); AS Sampler	1 vial (1.5 ml; 168 mM; buffered L-amino acid solution)
11	Leucine (Leu, L); AS Sampler	1 vial (1.5 ml; 140 mM; buffered L-amino acid solution)
12	Lysine (Lys, K); AS Sampler	1 vial (1.5 ml; 168 mM; buffered L-amino acid solution)
13	Methionine (Met, M); AS Sampler	1 vial (1.5 ml; 168 mM; buffered L-amino acid solution)
14	Phenylalanine (Phe, F); AS Sampler	1 vial (1.5 ml; 168 mM; buffered L-amino acid solution)
15	Proline (Pro, P); AS Sampler	1 vial (1.5 ml; 168 mM; buffered L-amino acid solution)
16	Serine (Ser, S); AS Sampler	1 vial (1.5 ml; 168 mM; buffered L-amino acid solution)

Vial	Label	Contents and function
17	Threonine (Thr, T); AS Sampler	1 vial (1.5 ml; 168 mM; buffered L-amino acid solution)
18	Tryptophan (Trp, W); AS Sampler	1 vial (1.5 ml; 168 mM; buffered L-amino acid solution)
19	Tyrosine (Tyr, Y); AS Sampler	1 vial (1.5 ml; 168 mM; buffered L-amino acid solution)
20	Valine (Val, V); AS Sampler	1 vial (1.5 ml; 168 mM; buffered L-amino acid solution)
21	DTT (1,3-Dithiothreitol); AS Sampler	5 vials (0.8 ml; 40 mM DTT)

Additional materials

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

- To perform protein synthesis reactions with RTS 100, RTS 500 ProteoMaster, or RTS 9000 *E. coli* HY Kits , the Eppendorf® ThermoMixer® C (or Eppendorf® Thermomixer Comfort) is required
- Pipets 0–10 µl, 10–200 µl, 200–1,000 µl, graduated 10 ml
- Pipet tips autoclaved at 121°C for 20 min
- Labeled or modified amino acids, for combination with the unlabeled amino acids provided with the RTS Amino Acid Sampler, must be obtained separately. The stock solution preparation for each labeled amino acid is described in Protocol 1, page 11.

One of the following kits must also be supplied by the user. The RTS Amino Acid Sampler is designed for use with:

- RTS 500 ProteoMaster *E. coli* HY Kit (cat. no. BR1400201)
- RTS 9000 *E. coli* HY Kit (cat. no. BR1400301)

It can also be used with:

- RTS 100 *E. coli* HY Kit (cat. no. BR1400101)

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 Amino Acid Sampler is shipped on dry ice.

The RTS Amino Acid Sampler and components should be stored at –15 to –25°C and are stable 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 10 freeze–thaw cycles.

Vials containing DTT (Vial 21) should only be opened a maximum of twice. The content should be aliquoted according to the volumes needed for the reactions.

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.

Vial 18 contains a solution with a pH of 1. Vial 19 contains a solution with a pH of 13. Both vials should be handled with care (wear appropriate eye and skin protection to prevent exposure). The usual precautions taken when handling chemicals should be observed. Reagents can be disposed of in waste water in accordance with local regulations. In case of eye contact, flush eyes immediately with water. If the undiluted content of vial 18 or vial 19 comes into contact with eyes, seek the advice of an ophthalmologist. In case of skin contact, wash off with water. In case of ingestion, seek medical advice.

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 can be obtained 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 Rapid Translation System (RTS) workflow (Figure 1) combines a series of new technologies for efficient and optimized protein expression. They overcome the limitations that often restrict the use of cell-free systems. These innovations include software-based template optimization, generation of stable expression templates without cloning, optimization of *in vitro* expression conditions and high yield *in vitro* expression, and an optimized lysate biochemistry.

The RTS Amino Acid Sampler provides the basis for the expression of labeled proteins using RTS 100, 500 ProteoMaster, or 9000 *E. coli* HY expression kits. The sampler allows the preparation of a customized amino acid mixture in which single amino acids can be easily exchanged with labeled ones. Using this customized amino acid mixture in RTS *E. coli* HY expression kits, the expressed protein can be specifically labeled for structure analysis (e.g. NMR, X-ray crystallography).

Advantages of the RTS Amino Acid Sampler

Protein labeling plays an important role in structural biology research. Methods and techniques used to analyze protein structures usually require labeling of the respective protein [1–3].

Cell-free protein synthesis has been shown to have exceptional advantages when proteins must be labeled for structural analysis [4, 5]:

- the protein of interest is expressed from an exogenous plasmid template and is the only protein produced and labeled
- the labeled amino acid can be applied directly to the reaction without penetrating the cellular membrane
- the translation reaction is performed in a small volume, requiring relatively low amounts of labeled amino acids
- no expensive media are needed since cell cultivation is not required
- amino acid metabolism in a cell-free system is significantly lower compared to cellular systems. Therefore, protein labeling is more specific and effective than in cell-based expression systems

Using the RTS *E. coli* HY expression kits with the RTS Amino Acid Sampler and selected labeled amino acid(s), it is possible to label almost each amino acid in the protein of interest without significant scrambling. For limitations regarding the incorporation of labeled amino acids, see the Section immediately below.

At present, all twenty amino acids, except for Glu and Gln, can be specifically incorporated into the expressed proteins. Some scrambling is observed with Ser, Asp, and Asn.

Description of procedure

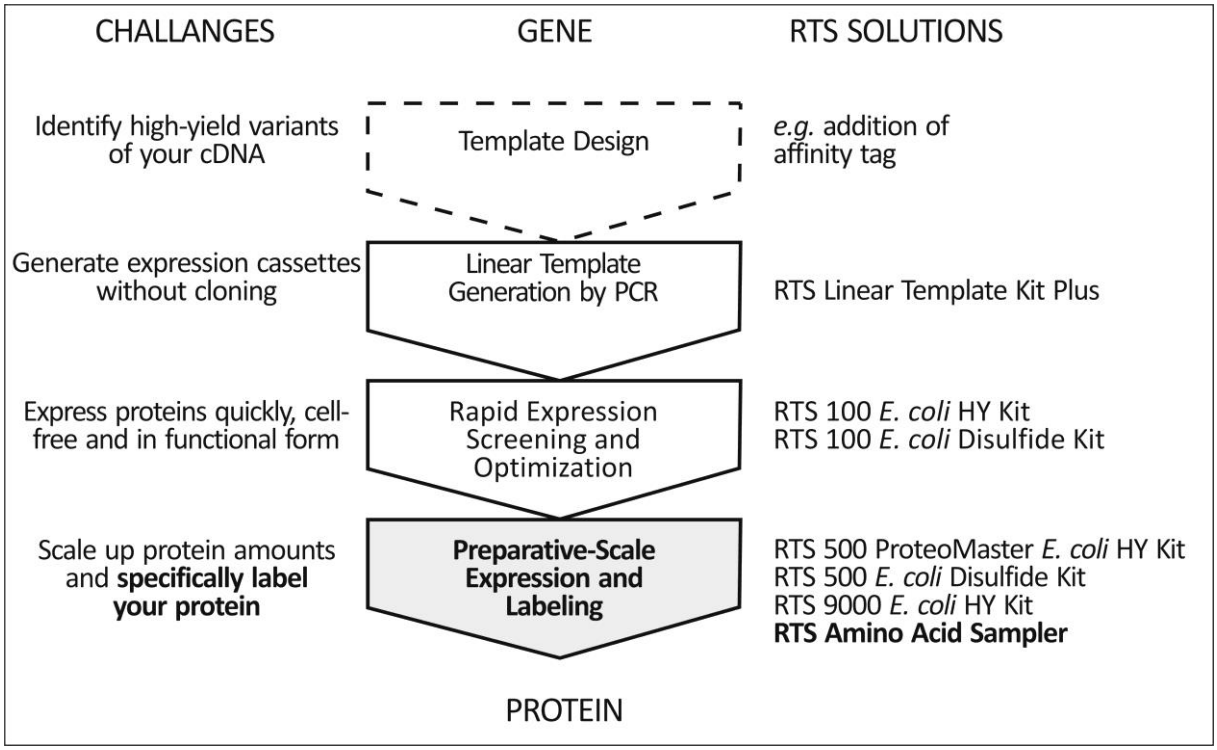

Figure 1. Integration of the RTS Amino Acid Sampler into the RTS workflow.

Protocol 1: Preparation of labeled amino acid stock solutions

All RTS *E. coli* HY expression kits are based on an *E. coli* lysate, and have expression restrictions for certain protein classes. To ensure the protein of interest is successfully expressed in RTS, start performing a small-scale test expression with the RTS 100 *E. coli* HY Kit (cat. no. BR1400101) – without protein labeling. The RTS 100 *E. coli* HY Kit allows the rapid production of up to 20 µg of protein in a 50 µl reaction.

Once positive results are obtained, scale-up the protein expression using the RTS 500 ProteoMaster *E. coli* HY Kit (cat. no. BR1400201). If expression yields are sufficient for the structural analysis, use this kit in combination with the RTS Amino Acid Sampler and the desired labeled amino acid(s) to express the labeled protein. If amounts of up to 50 mg of protein are required, proteins can be expressed and labeled using the RTS 9000 *E. coli* HY Kit (cat. no. BR1400301).

For detailed information about the RTS Kits, refer to the corresponding user manuals, or visit www.biotechrabbit.com.

Equipment and reagents required

- Eppendorf® ThermoMixer® C (or Eppendorf® Thermomixer Comfort)
- Calibrated pipets
- RNase-free plastic and glassware

Reagent notes

- Thaw the contents of the RTS Amino Acid Sampler at room temperature
- Dissolve precipitated amino acids by incubating the solutions for 5–10 minutes at 37°C
- Prepare the amino acid mixture directly prior to use since storage is only possible for up to four hours (15–25°C or 4°C). Prepared amino acid mixtures cannot be stored overnight at any temperature.

Procedure

Using the cell-free expression system RTS, each of the 20 amino acids (except for Glu and Gln) can be specifically incorporated into the expressed protein. When incorporating Ser, Asp, and Asn, scrambling may be observed. Labeled or modified amino acids are not provided with the RTS Amino Acid Sampler Kit.

To combine the labeled amino acids of choice with the unlabeled amino acids provided in the RTS Amino Acid Sampler, a 168 mM stock-solution preparation of the labeled amino acid is recommended (except Leu: 140 mM). Prepare the required labeled amino acid stock solution(s) as follows:

- Ala, Arg, Asn, Asp, Gly, His, Ile, Lys, Ser, Pro, Thr, and Val: Dissolve the amino acid in the Reconstitution Buffer of the respective RTS *E. coli* HY Kit. Asn requires a prolonged time period for complete dissolution

- Leu: Prepare only a 140 mM stock solution using the Reconstitution Buffer of the respective RTS *E. coli* HY Kit
- Met and Cys: Dissolve the amino acid in the Reconstitution Buffer of the respective RTS *E. coli* HY Kit, but add DTT (final concentration 4 mM and 8 mM, respectively)
- Tyr: Dissolve in 60 mM Hepes, pH 13; 0.7 M KOH
- Trp: Dissolve in 60 mM Hepes, pH 1; 0.7 M HCl
- Phe: Dissolve in 60 mM Hepes; pH 7.45 and treat with ultrasound for complete dissolution
- Labeled Glu and Gln cannot be incorporated into the expressed protein using the current RTS kits

Protocol 2: Preparation of the amino acid labeling mixture

The RTS Amino Acid Sampler provides each amino acid as a separate stock solution. The combination of the Sampler with the prepared stock solutions of labeled amino acids (Protocol 1, page 11) allows the preparation of a customized amino acid mixture in which single amino acids can be exchanged for labeled amino acids. Using this labeling mixture in the RTS expression reaction, proteins can be specifically labeled for structural studies.

Procedure

1. Prepare the working amino acid solutions according to Table 1.

Table 1. Preparation of working solutions

Component	Preparation of working solution	For use in
Amino acid stock solution (Vials 1–20)	Prepare the customized amino acid labeling mix by combining the stock solutions of the RTS Amino Acid Sampler with the prepared stock solutions of the labeled amino acids (Protocol 1, page 11).	This protocol
DTT (Vial 21)	→ Ready-to-use solution → Open each vial a maximum of twice. Aliquot according to the reactions needed → Repeated freezing and thawing will not affect performance	Protocol 3, page 15

Use a fresh pipet tip for each amino acid and an RNase-free tube for preparation of the mix.

To avoid precipitation, Trp, Tyr, and Leu should be added last.

2. After pipetting all 20 amino acids, the solution is thoroughly mixed by vortexing. The amino acid labeling mix is now ready to be added to the reaction and feeding solution of the RTS reaction (Protocol 3, page 15).

Table 2. Pipetting scheme for preparation of amino acid labeling mixture

	RTS 100 *E. coli* HY Kit	RTS 500 *E. coli* HY Kit	RTS 500 ProteoMaster *E. coli* HY Kit	RTS 9000 *E. coli* HY Kit
Number of reactions	5	1	1	1
Amount per amino acid	3 µl	37.5 µl	150 µl	1.5 ml
Addition of Reconstitution Buffer	–	2.25 ml	–	–
Final amino acid mix volume	60 µl	3 ml	3 ml	30 ml

Use a fresh pipet tip for each amino acid.

Add Trp, Tyr, and Leu last.

Use the Reconstitution Buffer provided in the respective RTS *E. coli* HY Kit.

Protocol 3: Protein synthesis reaction

Before starting

- The RTS Amino Acid Sampler is designed for use with the RTS 500 ProteoMaster *E. coli* HY or the RTS 9000 *E. coli* HY Kit. It can also be used with the RTS 100 *E. coli* HY Kit (for small-scale expression).
- Prepare the desired amino acid labeling mixture (Protocol 1, page 11)
- Reconstitute the required reaction components of the RTS expression kit
- Reconstitute only the needed number of bottles from the RTS *E. coli* HY Kit; use only the Reconstitution Buffer provided with the kit
- When combining the RTS *E. coli* HY kits with the RTS Amino Acid Sampler, the Amino Acids and the Methionine are not required (both are contained in every RTS *E. coli* HY Kit)

Procedure: Expression of labeled proteins using the RTS 100 *E. coli* HY Kit (cat. no. BR1400101)

1. Prepare stock solutions of the labeled amino acids for incorporation into the expressed protein, as described in Protocol 1, page 11.
2. Prepare the amino acid labeling mixture using the prepared labeled amino acid stock solutions and the unlabeled amino acid solutions provided with the RTS Amino Acid Sampler, as described in Protocol 2, page 13.
3. Reconstitute the following reaction components from the RTS 100 *E. coli* HY Kit, as described in kit manual (Table 2, Protocol 'Protein synthesis reaction', page 16).

   E. coli Lysate (bottle 1)

   Reaction Mix (bottle 2)
4. Set-up the RTS 100 *E. coli* HY reaction.

   Pipet the following components into one of the supplied reaction tubes:

   12 µl *E. coli* Lysate, reconstituted

   10 µl Reaction Mix, reconstituted

   12 µl of the prepared amino acid labeling mix (Step 2)

   1 µl DTT solution (vial 21 of the RTS Amino Acid Sampler)

   5 µl Reconstitution Buffer from the RTS 100 *E. coli* HY Kit

   0.5 µg DNA template in 10 µl water or TE buffer

   Carefully mix by rolling or gently shaking. Do not vortex!
5. Run the protein expression reaction as described in the manual for the RTS 100 *E. coli* HY Kit.

Procedure: Expression of labeled proteins using the RTS 500 ProteoMaster *E. coli* HY Kit (cat. no. BR1400201)

1. Prepare stock solutions of the labeled amino acids for incorporation into the expressed protein, as described in Protocol 1, page 11.
2. Prepare the amino acid labeling mixture using the prepared labeled amino acid stock solutions and the unlabeled amino acid solutions provided with the RTS Amino Acid Sampler, as described in Protocol 2, page 13.
3. Reconstitute the following reaction components from the RTS 500 ProteoMaster *E. coli* HY Kit, as described in the manual according to Table 5 in Protocol 4 'Production of labeled proteins for NMR spectroscopy' on page 22.

   E. coli Lysate (bottle 1)

   Reaction Mix (bottle 2)

   Feeding Mix (bottle 3)
4. Set-up the RTS 500 ProteoMaster *E. coli* HY reaction.

   Prepare the following working solutions:

   Reaction solution:

   Add 0.225 ml of the reconstituted Reaction Mix, 0.27 ml of prepared amino acid labeling mix (from step 2), and 30 µl DTT (vial 21, RTS Amino Acid Sampler) to the reconstituted *E. coli* Lysate. Add 10–15 µg of DNA template in a maximum volume of 50 µl. Mix by rolling or gently shaking. Do not vortex!

   Feeding Solution:

   Add 2.65 ml of the prepared amino acid labeling mix (from step 2) and 0.3 ml DTT (vial 21, RTS Amino Acid Sampler) to the reconstituted Feeding Mix. Mix by rolling or gentle shaking.
5. Run the protein expression reaction as described in the manual for the RTS 500 ProteoMaster *E. coli* HY Kit.

Procedure: Expression of labeled proteins using the RTS 9000 *E. coli* HY Kit (cat. no. BR1400301)

1. Prepare stock solutions of the labeled amino acids for incorporation into the expressed protein, as described in Protocol 1, page 11.
2. Prepare the amino acid labeling mixture using the prepared labeled amino acid stock solutions and the unlabeled amino acid solutions provided with the RTS Amino Acid Sampler, as described in Protocol 2, page 13.
3. Reconstitute the following reaction components from the RTS 9000 *E. coli* HY Kit, as described in the manual according to Table 6 in Protocol 4 'Production of labeled proteins for NMR spectroscopy' on page 23.

   E. coli Lysate (vial 1)

   Reaction Mix (vial 2)

   Feeding Mix (vial 3)
4. Set-up the RTS 9000 *E. coli* HY reaction.

   Prepare the following working solutions:

   Reaction solution:

   Add 2.2 ml of the reconstituted Reaction Mix, 2.7 ml of prepared amino acid labeling mix (from step 2), and 300 µl DTT (vial 21, RTS Amino Acid Sampler) to the reconstituted *E. coli* Lysate. Add 120–180 µg of DNA template in a maximum volume of 500 µl. Mix by rolling or gently shaking. Do not vortex!

   Feeding Solution:

   Add 26 ml of the prepared amino acid labeling mix (from step 2) and 3 ml DTT (vial 21, RTS Amino Acid Sampler) to the reconstituted Feeding Mix. Mix by rolling or gentle shaking.
5. Run the protein expression reaction as described in the manual for the RTS 9000 *E. coli* HY Kit.

Supporting information

Short protocol

Use the following short protocols only if you are familiar with the standard procedures described in this manual:

1. Prepare 168 mM (Leu: 140 mM) stock solutions of labeled amino acids.
2. Prepare the amino acid labeling mix by combining the RTS Amino Acid Sampler and the labeled amino acid stock solutions.

   Use the following pipetting scheme for preparation of the labeling mixture.

	RTS 100 *E. coli* HY Kit	RTS 500 *E. coli* HY Kit	RTS 500 ProteoMaster *E. coli* HY Kit	RTS 9000 *E. coli* HY Kit
Number of reactions	5	1	1	1
Amount per amino acid	3 µl	37.5 µl	150 µl	1.5 ml
Addition of Reconstitution Buffer	–	2.25 ml	–	–
Final amino acid mix volume	60 µl	3 ml	3 ml	30 ml

Use a fresh pipet tip for each amino acid.

Use the Reconstitution Buffer provided in the respective RTS *E. coli* HY Kit.

3. Set up the RTS protein reaction for the relevant kit, as below.

Kit	Reaction Solution
RTS 100 *E. coli* HY	Pipet the following components into one of the supplied reaction tubes: → 12 µl *E. coli* Lysate, reconstituted → 10 µl Reaction Mix, reconstituted → 12 µl of the prepared amino acid labeling mix → 1 µl DTT solution (vial 21) → 5 µl Reconstitution Buffer → 0.5 µg DNA template in 10 µl water or TE buffer Carefully mix by rolling or gently shaking. Do not vortex!

	Reaction Solution	Feeding Solution
RTS 500 *E. coli* HY	Add to the reconstituted *E. coli* Lysate: → 0.225 ml reconstituted Reaction Mix → 0.27 ml prepared amino acid labeling mix → 30 µl DTT (vial 21) → 10–15 µg DNA template (max. volume 50 µl) Mix by rolling or gently shaking. Do not vortex!	Add to the reconstituted Feeding Mix: → 2.65 ml prepared amino acid labeling mix → 0.3 ml DTT (vial 21). Mix by rolling or gentle shaking.
RTS 500 ProteoMaster *E. coli* HY	Add to the reconstituted *E. coli* Lysate: → 0.225 ml reconstituted Reaction Mix → 0.27 ml prepared amino acid labeling mix → 30 µl DTT (vial 21) → 10–15 µg DNA template (max. volume 50 µl) Mix by rolling or gently shaking. Do not vortex!	Add to the reconstituted Feeding Mix: → 2.65 ml prepared amino acid labeling mix → 0.3 ml DTT (vial 21). Mix by rolling or gentle shaking
RTS 9000 *E. coli* HY	Add to the reconstituted *E. coli* Lysate: → 2.2 ml reconstituted Reaction Mix → 2.7 ml prepared amino acid labeling mix → 300 µl DTT (vial 21) → 120–180 µg DNA template (max. volume 500 µl) Mix by rolling or gently shaking. Do not vortex!	Add to the reconstituted Feeding Mix: → 26 ml prepared amino acid labeling mix → 3 ml DTT (vial 21) Mix by rolling or gentle shaking

Typical results

Use of the RTS Amino Acid Sampler in RTS 100, 500, and 9000 *E. coli* HY Kits

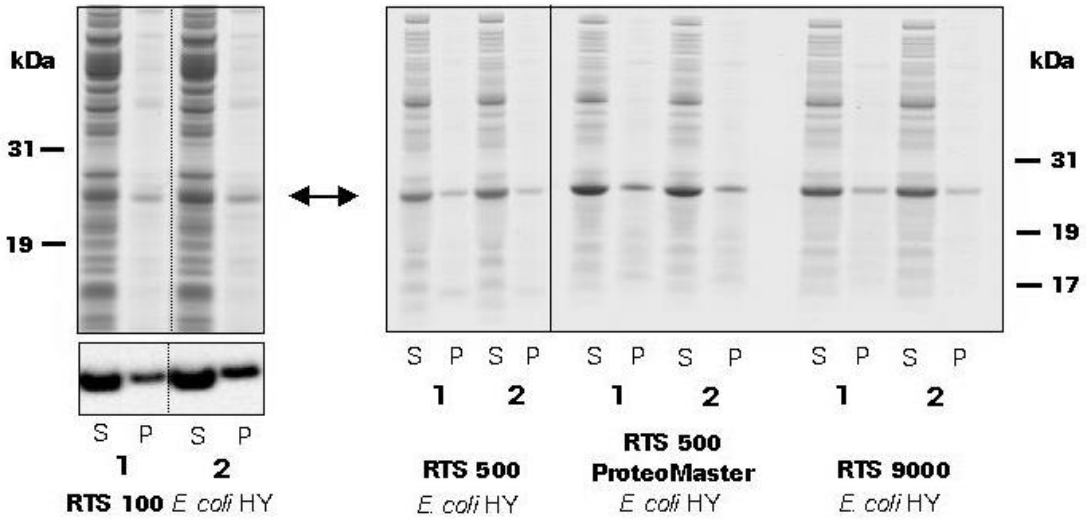

Figure 2. Expression of CAT using RTS 100, 500, and 9000 reactions in combination with the RTS Amino Acid Sampler.

CAT was expressed in the different RTS reactions using the ready-to-use amino acid mixture provided with the respective RTS kit (1) or by using the amino acid stock solutions provided with the RTS Amino Acid Sampler (2). Reactions were performed according to the procedures described in this manual and analyzed on Coomassie®-stained SDS gels. The RTS 100 HY reaction was also analyzed by western blot using an Anti-His_6 antibody. **S**: supernatant; **P**: pellet.

^{2}H, ^{15}N complete protein labeling using the RTS 500 ProteoMaster *E. coli* HY Kit

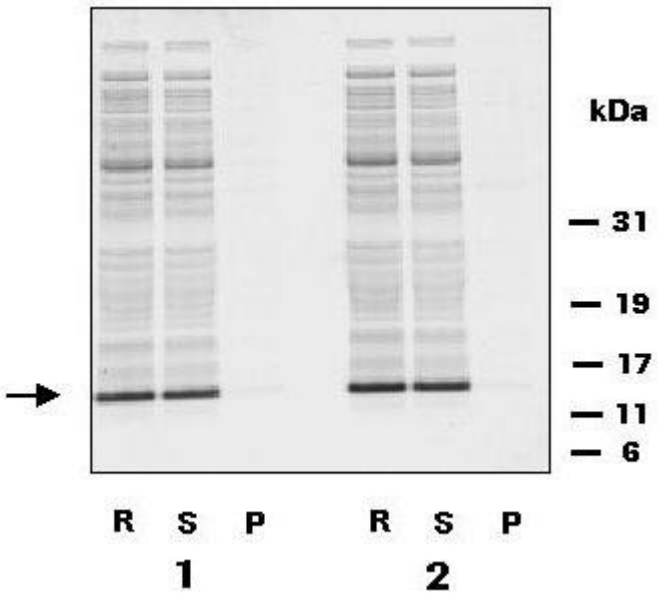

Figure 3. Comparison of the expression of unlabeled and completely ^{2}H, ^{15}N-labeled SH3 domain (8.5 kDa).

RTS 500 ProteoMaster *E. coli* HY reactions were performed using the unlabeled amino acid mixture provided with the kit (1) and a ^{2}H, ^{15}N-labeled amino acid mixture (2). Since the ^{2}H, ^{15}N-labeled amino acid mixture (Spectra Gases, Inc., Branchburg, NY, USA) contains only low concentrations of His, Ile, Met, Trp, and Tyr, these amino acids were supplemented to a final concentration of 2 mM. After 24 hours, reactions were analyzed on a Coomassie-stained SDS-PAGE. **R**: Total expression reaction; **S**: Supernatant; **P**: Pellet.

Selective labeling of protein using RTS

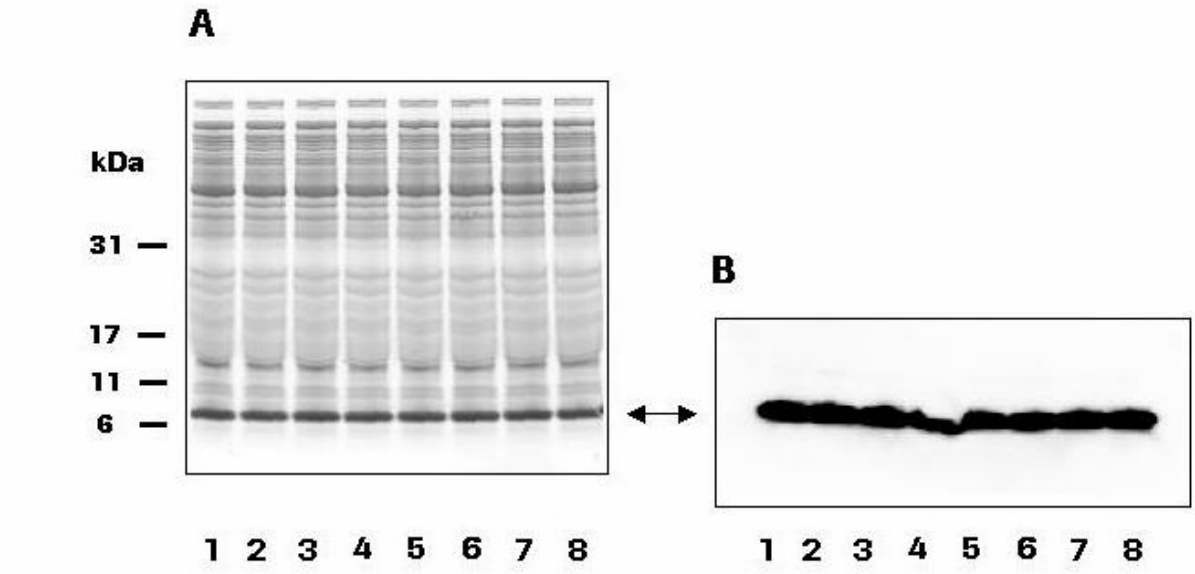

Figure 4. RTS 500 *E. coli* HY expression of uniformly and selectively labeled SH3 domain (8.5 kDa).

The SH3 domain was expressed in a RTS 500 *E. coli* HY reaction using unlabeled amino acids (lane 1), a mixture of all ^{15}N-labeled amino acids (lane 2) and single ^{15}N-amino acids (lanes 3–8). Reactions were analyzed on a Coomassie-stained SDS-PAGE (A) and a western blot (B) using an Anti-His_6 antibody. No differences in expression yields of unlabeled, completely labeled, and selectively labeled protein were observed. **1**: Unlabeled SH3; **2**: Completely ^{15}N-labeled SH3; **3– 8**: Selectively labeled SH3 using ^{15}N-labeled Val (3), Tyr (4), Trp(5), Thr (6), Pro (7), and Phe (8).

References

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2. Gardner, K.H., Kay, L.E. (1998). Annu. Rev. Biophys. Biomol. Struct. 27, 357.
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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.

Observation	No protein was produced
Possible cause	The protein is difficult to express in RTS
Resolving	First, test expression in the RTS 100 *E. coli* HY Kit (cat. no. BR1400101). Follow the recommendations provided with the kit for increasing the success rate.

Observation	No protein was produced, although RTS 100 protein production was positive
Possible cause	The amino acid labeling mix is missing one or more amino acids
Resolving	Repeat the experiment using freshly prepared mixtures. Compare the expression results using the amino acids provided in the RTS 500 ProteoMaster *E. coli* HY Kit (cat. no. BR1400201).

Observation	Some amino acids show precipitation after thawing
Resolving	Warm vials to 37–42°C. Precipitates must be dissolved.

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 *E. coli* HY Kit	24 reactions	BR1400101
RTS 100 *E. coli* HY Kit	96 reactions	BR1400102
RTS 500 ProteoMaster *E. coli* HY Kit	5 reactions	BR1400201
RTS 9000 *E. coli* HY Kit	1 reaction	BR1400301
RTS 100 *E. coli* Disulfide Kit	24 reactions	BR1400401
RTS 500 *E. coli* Disulfide Kit	5 reactions	BR1400501
RTS pIVEX *E. coli* 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