RTS™ DnaK Supplement Short Instruction

For supplementation of RTS cell-free protein expression systems

Product description

The RTS DnaK Supplement provides the components necessary to supplement cell-free protein expression systems.

The product can be used with the RTS 100 *E. coli* HY, RTS 500 ProteoMaster *E. coli* HY and RTS 9000 *E. coli* HY Kits.

Product limitations

RTS DnaK Supplement 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 DnaK Supplement	Contents and use
Ordering number	BR1401601
DnaK Mix; DnaK Supplement (purple cap)	→ 5 vials; 180 µl per vial → Contains DnaK, DnaJ, and GrpE as components of a bacterial lysate
Energy Mix; DnaK Supplement (colorless cap)	→ 1 vial (1.5 ml) → Contains ATP

Additional materials

To perform the protocols described in this manual, one of the following additional kits (available from biotechrabbit) must be provided by the user:

- RTS 100 *E. coli HY Kit* (cat. no. BR1400101)
- RTS 500 ProteoMaster *E. coli* HY Kit (cat. no. BR1400201)
- RTS 9000 *E. coli* HY Kit (cat. no. BR1400301)

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 DnaK Supplement is shipped on dry ice.

The RTS DnaK Supplement should be stored at –20°C and is stable until the expiration date printed on the label. Up to three freeze–thaw cycles do not decrease activity. After thawing, RTS DnaK Supplement is stable for 4 hours at 4°C.

The Energy Mix should be stored at –20°C and remains stable for up to 20 freeze–thaw cycles. For convenience, the Energy Mix may be aliquoted.

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 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) is a flexible and scalable tool for cell-free protein expression. Reaction conditions can be easily adapted in a protein-specific manner by adding, for example, co-factors, ligands, or detergents to the reaction mixture.

An important example of this is the addition of chaperones in order to increase the amount of correctly folded and/or soluble recombinant protein. DnaK (heat shock protein 70, hsp70), together with DnaJ (Hsp 40) and GrpE, is one of the key chaperone systems in *E. coli*. *In vivo* DnaK is involved in:

- prevention of aggregation and refolding of misfolded proteins
- mediation of degradation of unstable proteins by proteases
- modulation of heat shock response
- protein translocation and other processes

For its folding activity, the DnaK protein depends on ATP/ADP-conversion and on the DnaJ and GrpE co-chaperones (Figure 1)

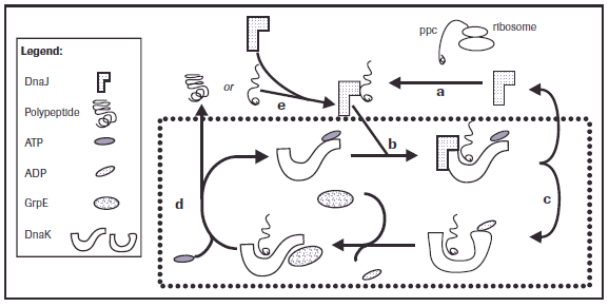

Figure 1. Schematic of protein folding mediated by the DnaK-chaperone system.

a) The nascent polypeptide chain (ppc) is bound by the DnaJ co-chaperone. b) This complex is recognized by DnaK in its ATP-bound state. c) Subsequently, the DnaJ is released and the ATP is hydrolyzed to give the substrate-DnaK complex. d) Finally, GrpE stimulates ADP dissociation and enables DnaK to bind ATP. The binding of ATP releases the GrpE and the polypeptide. e) If the polypeptide is still not folded correctly, it may undergo another cycle.

Mutagenesis studies have revealed which domain of DnaK interacts with the protein substrate and with the co-chaperones DnaJ or GrpE, respectively. In addition, peptide scan experiments have shown that DnaK binds to stretches of hydrophobic amino acid residues. However, even with these findings, it remains difficult to predict whether or not a given protein will be recognized as substrate for the DnaK chaperone system.

In contrast to the GroE chaperone system (cat. no. BR1401701), the application of DnaK is not limited by the size of the target proteins to which it binds. In addition, it can also serve as an aggregation inhibitor that binds unfolded protein or may transfer it to other chaperones, such as the GroE or the Hsp90 systems. A transfer of misfolded proteins to *E. coli* proteases may also occur.

Applications

To optimize the yield and solubility of proteins expressed in the RTS, biotechrabbit recommends to first express the protein in the RTS 100 *E. coli* HY Kit, in the presence or absence of different chaperones and/or detergents.

Once a positive effect is observed in RTS 100 *E. coli* HY batch reactions (50 µl), the same conditions can, in principle, also be applied to continuous exchange cell-free (CECF) reactions (performed, for example, using the RTS 500 ProteoMaster *E. coli* HY Kit).

However, since expression rates under CECF conditions are significantly higher than in batch mode, added DnaK may reach a point where it is insufficient for the quantity of protein produced (this effect is dependent on each specific substrate protein). Therefore, when using DnaK under CECF conditions, biotechrabbit recommends running kinetic studies by taking samples after 2, 4, and 6 hours and analyzing the respective ratio of soluble and insoluble protein.

Specificity

The DnaK system (DnaK, DnaJ, GrpE) is a prokaryotic chaperone system. The system has no substrate limitation regarding protein size and, in principle, can act on all proteins. However, it is impossible to predict exactly to what extent a protein's folding can be improved by DnaK. As with *in vivo* situations (expression in intact *E. coli* cells), DnaK may also present the protein to proteases, resulting in a reduction of total protein yield.

Protocol 1: Supplementing RTS 100 *E. coli* HY reactions

1. Reconstitute the reaction components according to Table 1.

Table 1. Reaction components

Solution	Contents	Reconstitution procedure	For use in
1	*E. coli* Lysate; 100 *E.coli* (Bottle 1, red cap)	Reconstitute the lyophilizate with 0.36 ml Reconstitution Buffer (bottle 5), mix carefully by rolling or gentle shaking. Do not vortex!	Step 2 Solution 7
2	Reaction Mix; 100 *E.coli* (Bottle 2, green cap)	Reconstitute the lyophilizate with 0.30 ml Reconstitution Buffer (bottle 5), mix by rolling or shaking.	Step 2 Solution 7
3	Amino Acids; 100 *E.coli* (Bottle 3, brown cap)	Reconstitute the lyophilizate with 0.36 ml Reconstitution Buffer (bottle 5), mix by rolling or shaking.	Step 2 Solution 7
4	Methionine; 100 *E.coli* (Bottle 4, yellow cap)	Reconstitute the lyophilizate with 0.33 ml Reconstitution Buffer (bottle 5), mix by rolling or shaking.	Step 2 Solution 7
5	Reconstitution Buffer; 100 *E.coli* (Bottle 5, white cap)	→ 1.6 ml → Ready-to-use solution → The solution is stable at 2 to 8°C, but can also be stored at –15 to –25°C	Solutions 1, 2, 3, and 4

All reconstituted solutions should be clear, with the exception of the *E. coli* lysate, which remains cloudy.

Numbers refer to the bottle numbers in the RTS 100 *E. coli* HY Kit.

2. Prepare the working solution according to Table 2.

   Note: Differences between the standard (chaperone-free) procedures and those using DnaK Supplement are marked in **bold**.

Table 2. Working solution

Solution	Contents	Preparation of working solution for one 50 µl reaction	For use in
7	Reaction Solution	Into one of the reaction tubes supplied, pipet the following components: → 12 µl *E. coli* Lysate → 10 µl Reaction Mix → 12 µl Amino Acids → 1 µl Methionine → 5 µl Reconstitution Buffer → **5–8 µl DnaK Mix** → **1.2 µl Energy Mix** → 0.5 µg circular DNA template or 0.5 µg linear template in 10 µl water or TE buffer → **Note: The final volume will be slightly higher than in the standard RTS 100 *E. coli* HY protocol** → **Note: For control reactions without DnaK Supplement replace the volume of DnaK Supplement with the same volume of Reconstitution 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! → Run the reaction according to the RTS 100 *E. coli* HY Kit Manual	Running an experiment see RTS 100 *E. coli* HY Kit Manual

Protocol 2: Supplementing RTS 500 ProteoMaster *E. coli* HY reactions

Before starting

→ When supplementing RTS 500 ProteoMaster *E. coli* HY reactions with chaperones, ensure that the *E. coli* lysate (bottle 1) is reconstituted in only **0.34 ml** (instead of 0.575 ml) of reconstitution buffer. Otherwise the DnaK supplement cannot be added because of volume constraints.

Protocol

1. Reconstitute the reaction components according to Table 3.

   Note: Differences between the standard (chaperone-free) procedures and those using DnaK Supplement are marked in **bold**.

Table 3. Reaction components

Solution	Contents	Reconstitution procedure	For use in
1	*E. coli* Lysate; 500 PM *E. coli* (Bottle 1, red cap)	Reconstitute the lyophilizate with **0.34 ml** Reconstitution Buffer (bottle 6), mix carefully by rolling or gentle shaking. Do not vortex! **Important note**: Centrifuge the reconstituted lysate at 14,000 rpm for 10 min. Separate the supernatant from the pellet and discard the pellet.	Step 2 Solution 8
2	Reaction Mix; 500 PM *E. coli* (Bottle 2, green cap)	Reconstitute the lyophilizate with 0.25 ml Reconstitution Buffer (bottle 6), mix by rolling or shaking.	Step 2 Solution 8
3	Feeding Mix; 500 PM *E. coli* (Bottle 3, blue cap)	Reconstitute the lyophilizate with 8.1 ml Reconstitution Buffer (bottle 6), mix by rolling or shaking.	Step 2 Solution 7
4	Amino Acids; 500 PM *E. coli* (Bottle 4, brown cap)	Reconstitute the lyophilizate with 3 ml Reconstitution Buffer (bottle 6), mix by rolling or shaking.	Step 2 Solutions 7 and 8
5	Methionine; 500 PM *E. coli* (Bottle 5, yellow cap)	Reconstitute the lyophilizate with 1.8 ml Reconstitution Buffer (bottle 6), mix by rolling or shaking.	Step 2 Solutions 7 and 8
6	Reconstitution Buffer; 500 PM *E. coli* (Bottle 6, white cap)	→ Ready-to-use solution → The solution is stable at 2 to 8°C, but can also be stored at –15 to –25°C	Solutions 1, 2, 3, 4, and 5

Reconstitution of the *E. coli* lysate will result in a slightly turbid, yellowish solution. Reconstitution of all other lyophilizates should result in clear solutions.

Numbers refer to the bottle numbers in the RTS 500 ProteoMaster *E. coli* HY Kit.

2. Prepare the working solutions according to Table 4.

   Note: Differences between the standard (chaperone-free) procedures and those using DnaK Supplement are marked in **bold**.

Table 4. Working solutions

Solution	Content	Preparation of working solution	For use in
7	Feeding Solution	Add 2.65 ml reconstituted Amino Acids (solution 4) and 0.3 ml reconstituted Methionine (solution 5) to Feeding Mix (solution 3). Finally, **add 66 µl Energy Mix** and mix by rolling or gentle shaking. Total volume of Feeding Solution is 11 ml.	Running an experiment, see RTS 500 ProteoMaster *E. coli* HY Kit Manual
8	Reaction Solution	To the content of solution 1 (*E. coli* Lysate), add 0.225 ml reconstituted Reaction Mix (solution 2), 0.27 ml reconstituted Amino Acids (solution 4) and 30 µl reconstituted Methionine (solution 5). **Add 180 µl DnaK Mix and 6 µl Energy Mix**. Add 10–15 µg of the DNA template in a maximum volume of 50 µl. Mix carefully by rolling or gentle shaking (do not vortex). Total volume of reaction solution is 1.1 ml.	Running an experiment, see RTS 500 ProteoMaster *E. coli* HY Kit Manual

Important note: biotechrabbit recommends running DnaK supplemented CECF reactions at 25°C, rather than 30°C, in order to allow the chaperones more time for folding the synthesized protein.

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