

RTS™ GroE Supplement Short Instruction

For supplementation of cell-free protein expression systems

Product description

The RTS GroE 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 GroE 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 GroE Supplement	Contents
Ordering number.	BR1401701
GroEL and GroES	5 vials; 125 µl

Additional materials

To perform the protocols described in this manual, one of the following additional kits 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 GroE Supplement is shipped on dry ice.
The RTS GroE Supplement should be stored at –15 to –25°C and is stable until the expiration date printed on the label.
Up to three freeze–thaw cycles do not decrease activity.

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

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 chemicals or protein factors 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 product.

GroEL and GroES (together called GroE) are cytoplasmic chaperonins of *E. coli*. It is believed that they are involved in the de novo folding of 10–30% of all cytoplasmic proteins. GroEL (57 kDa, monomer) is arranged in two heptameric rings, which in turn form a cylindrical complex. GroES (10.5 kDa, monomer) forms dome-shaped heptameric rings which cap the GroEL cylinder on one or both ends. The size of the cylinder allows proteins with a molecular mass up to 60 kDa to be substrates for the GroE system. Although it is not predictable whether or not a particular protein is a substrate for GroE, the presence of GroE during cellular or

cell-free protein synthesis has been reported to lead to higher amounts of correctly folded and active product.

In order to efficiently optimize the RTS reaction conditions and to study the effect of chaperonin addition, biotechrabbit recommends to first use the RTS 100 *E. coli* HY Kit (biotechrabbit). Once a positive effect is observed in the 50 µl reaction volume of RTS 100 *E. coli* HY Kit, the same conditions can be applied to the 1 ml reaction volume of the RTS 500 *E. coli* HY Kit and the RTS 500 ProteoMaster *E. coli* HY Kit (biotechrabbit).

Specificity

The GroE system (GroEL/GroES) is a prokaryotic chaperone system. It can fold proteins in the range between 20 and 60 kDa. Smaller proteins are not recognized and larger proteins do not fit into the cavity.

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

1. Reconstitute the reaction components according to Table 1.
2. Prepare the working solution according to Table 2.

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

Table 1. Reaction components

Solution	Contents	Reconstitution procedure	For use in
1	<i>E. coli</i> Lysate; 100 <i>E. coli</i> (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 <i>E. coli</i> (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 <i>E. coli</i> (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 <i>E. coli</i> (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 <i>E. coli</i> (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.

Table 2. Working solution

Solution	Contents	Preparation of working solution for one 50 µl reaction	For use in
7	Reaction Solution	<p>Into one of the reaction tubes supplied, pipet the following components:</p> <ul style="list-style-type: none"> → 12 µl <i>E. coli</i> Lysate → 10 µl Reaction Mix → 12 µl Amino Acids → 1 µl Methionine → 6 µl GroE Supplement → 0.5 µg circular DNA template or 0.5 µg linear template in 10 µl water or TE buffer → <u>Note: The final volume will be slightly higher than in the standard RTS 100 <i>E. coli</i> HY protocol</u> → <u>Note: For control reactions without GroE Supplement replace the volume of GroE Supplement with the same volume of Reconstitution Buffer</u> → A premix of the following solutions without DNA is recommended for multiple parallel reactions: <ul style="list-style-type: none"> → <i>E. coli</i> Lysate; 100 <i>E. coli</i> (solution 1) → Reaction Mix; 100 <i>E. coli</i> (solution 2) → Amino Acids; 100 <i>E. coli</i> (solution 3) → Methionine; 100 <i>E. coli</i> (solution 4) → <u>GroE Supplement</u> → Mix carefully by rolling or gentle shaking; do not vortex! → Run the reaction according to the RTS 100 <i>E. coli</i> HY Kit Manual 	Running an experiment, see RTS 100 <i>E. coli</i> 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 0.525 ml) of reconstitution buffer. Otherwise the GroE 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 GroE Supplement are marked in **bold**.
2. Prepare the working solutions according to Table 4.
Note: Differences between the standard (chaperone-free) procedures and those using GroE Supplement are marked in **bold**.

Table 3. Reaction components

Solution	Contents	Reconstitution procedure	For use in
1	<i>E. coli</i> Lysate; 500 PM <i>E. coli</i> (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!	Step 2 Solution 8
2	Reaction Mix; 500 PM <i>E. coli</i> (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 <i>E. coli</i> (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 <i>E. coli</i> (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 <i>E. coli</i> (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 <i>E. coli</i> (Bottle 6, white cap)	<p>→ Ready-to-use solution</p> <p>→ The solution is stable at 2 to 8°C, but can also be stored at –15 to –25°C</p>	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.

Table 4. Working solutions

Solution	Contents	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). Mix by rolling or shaking. Total volume of Feeding Solution is 11 ml.	Running an experiment, see RTS 500 ProteoMaster <i>E. coli</i> HY Kit Manual
8	Reaction Solution	To the content of solution 1 (<i>E. coli</i> Lysate), add 0.225 ml reconstituted Reaction Mix (solution 2), 0.270 ml reconstituted Amino Acids (solution 4) and 30 µl reconstituted Methionine (solution 5). Add 125 µl GroE Supplement and 60 µl Reconstitution Buffer. Finally, add 10–15 µg DNA template in a maximum volume of 50 µl. Mix carefully by rolling or gentle shaking (do not vortex). Total volume 1.1 ml	Running an experiment, see RTS 500 ProteoMaster <i>E. coli</i> HY Kit Manual

Trademarks and Disclaimers

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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.