

# RTS GroE Supplement

Cat. No. 03 263 690 001

For five 1 ml reactions

Version January 2007

Store at -15°C to -25°C

## 1. Product overview

<b>Product description</b>	Solution, contains GroEL and GroES. The set includes 5 vials, 125 µl each, for use in the cell-free protein expression systems RTS 100 <i>E. coli</i> HY*, RTS 500 <i>E. coli</i> HY*.
<b>Stability</b>	Stable at -15 to -25°C. Freeze/thaw cycles up to three times do not decrease the activity.
<b>Specificity</b>	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.
<b>Safety information</b>	None of the bottles contain 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.

## 2. Background information

The Rapid Translation System is a flexible and scalable tool for cell-free protein expression. It can be considered as "open" system, meaning that the reaction conditions can easily be adapted in a protein-specific manner by adding chemicals or protein factors to the reaction mixture.

A very important example in this regard is the addition of chaperones in order to increase the amount of correctly folded and/or soluble product.

GroEL and GroES (the combination is named GroE) are cytoplasmic chaperonins of *E. coli* (1,2). It is believed that they are involved in the *de novo* folding of 10 - 30% of all cytoplasmic proteins (3). GroEL (57 kDa, monomer) is arranged in 2 heptameric rings which in turn form a cylindric 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 that a particular protein is a substrate for GroE, the presence of GroE during cellular or cell-free protein synthesis has been reported in many cases 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, we generally recommend to first use the RTS 100 *E. coli* HY Kit (Cat. No. 03 186 148 001). Once a positive effect is observed in the 50 µl reaction volume of RTS 100, the same conditions can be applied to the 1 ml reaction volume of the RTS 500 *E. coli* HY Kit (Cat. No. 03 246 817 001 and 03 246 949 001).

## 3. Application

**Note** In all protocols given below, differences between the standard (chaperonin-free) procedures and procedures using GroE Supplement are marked in **bold**.

### 3.1 Supplementing RTS 100 *E. coli* HY reactions

#### 3.1.1 Reconstitution of reaction components

Solution	Content	Reconstitution/Preparation of working solution	For use in
1	<i>E. coli</i> 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 3.1.2 • 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 3.1.2 • 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 3.1.2 • 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 3.1.2 • solution 7
5	Reconstitution Buffer Bottle 5, white	• 1.6 ml • Ready-to-use solution • The solution is stable at 2-8°C but can also be stored at -15 to -25°C	solutions 1, 2, 3, 4

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

### 3.1.2 Preparation of working solutions

Solution	Content	Reconstitution/ Preparation of working solution
7	Reaction Solution	<p>Into one of the supplied reaction tubes pipet the following components:</p> <ol style="list-style-type: none"> <li>1 12 µl <i>E. coli</i> Lysate</li> <li>2 10 µl Reaction Mix</li> <li>3 12 µl Amino Acids</li> <li>4 1 µl Methionine</li> <li>5 <b>6 µl of RTS GroE Supplement</b></li> <li>6 0.5 µg of the circular DNA or linear template in 10 µl of water or TE-buffer.</li> </ol> <ul style="list-style-type: none"> <li>• <b>Please note: the final volume will be slightly higher than in case of the standard RTS 100 <i>E. coli</i> HY protocol !</b></li> <li>• A premix of solutions 1–6 without DNA is recommended for multiple parallel reactions.</li> <li>• Mix carefully by rolling or gentle shaking.</li> <li>• <b>DO NOT VORTEX !</b></li> <li>• Run the reaction</li> </ul>

**Reaction set-up** Please refer to section 3.1.4 of the manual of Cat. No. 03 186 148 001.

### 3.2 Supplementing RTS 500 *E. coli* HY reactions

#### 3.2.1 Reconstitution of reaction components

**Before you start** When supplementing the RTS 500 *E. coli* HY kit reaction with chaperones, be sure the *E. coli* lysate (bottle 1) is reconstituted in only 0.34 ml of reconstitution buffer instead of 0.575 ml. This change in volume gives you the opportunity to add this supplement.

Solution	Content	Reconstitution/Preparation of working solution	For use in
1	<i>E. coli</i> Lysate Bottle 1, red cap	Reconstitute the lyophilizate with <b>0.340</b> ml of Reconstitution Buffer (bottle 6), mix carefully by rolling or gentle shaking. <b>DO NOT VORTEX!</b>	<ul style="list-style-type: none"> <li>• section 3.2.2</li> <li>• solution 8</li> </ul>
2	Reaction Mix Bottle 2, green cap	Reconstitute the lyophilizate with 0.25 ml of Reconstitution Buffer (bottle 6), mix by rolling or shaking.	<ul style="list-style-type: none"> <li>• section 3.2.2</li> <li>• solution 8</li> </ul>
3	Feeding Mix Bottle 3, blue cap	Reconstitute the lyophilizate with 8.1 ml of Reconstitution Buffer (bottle 6), mix by rolling or shaking.	<ul style="list-style-type: none"> <li>• section 3.2.2</li> <li>• solution 7</li> </ul>
4	Amino Acid Mix without Methionine Bottle 4, brown cap	Reconstitute the lyophilizate with 3 ml of Reconstitution Buffer (bottle 6), mix by rolling.	<ul style="list-style-type: none"> <li>• section 3.2.2</li> <li>• solution 7 and 8</li> </ul>
5	Methionine Bottle 5, yellow cap	Reconstitute the lyophilizate with 1.8 ml of Reconstitution Buffer (bottle 6), mix by rolling or shaking.	<ul style="list-style-type: none"> <li>• section 3.2.2</li> <li>• solution 7 and 8</li> </ul>
6	Reconstitution Buffer Bottle 6, white cap	<ul style="list-style-type: none"> <li>• Ready-to-use solution</li> <li>• The solution is stable at +2 to +8°C but can also be stored at –15 to –25°C</li> </ul>	solutions 1, 2, 3, 4, 5

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

### 3.2.2 Preparation of working solutions

Solution	Content	Reconstitution/Preparation of working solution	For use in
7	Feeding Solution	Add 2.65 ml of the reconstituted Amino Acid Mix without Methionine (solution 4) and 0.3 ml of reconstituted Methionine (solution 5) to solution 3. Mix by rolling or shaking. Total volume now will be 11 ml.	section 3.1.4 of the manual of Cat. Nos. 03 246 817 001 and 03 246 949 001.
8	Reaction Solution	To the content of solution 1 add 0.225 ml of the reconstituted Reaction Mix (solution 2), 0.27 ml of the reconstituted Amino Acid Mix without Methionine (solution 4) and 30 µl of reconstituted Methionine (solution 5). <b>Add 125 µl of RTS GroE supplement plus 60 µl Reconstitution buffer.</b> Finally, add 10–15 µg of DNA template in a maximum volume of 50 µl. Mix carefully by rolling or gentle shaking. Total volume 1.1 ml <b>DO NOT VORTEX!</b>	section 3.1.4 of the manual of Cat. Nos. 03 246 817 001 and 03 246 949 001.

**Reaction set-up** Please refer to section 3.1.4. of the manual of Cat. Nos. 03 246 817 001 (2 reactions pack size) or 03 246 949 001 (5 reactions pack size).

### 3.3 Supplementing RTS 500 *E. coli* Circular Template reactions

### 4. Example

#### 3.3.1 Reconstitution of reaction components

Solution	Content	Reconstitution/Preparation of working solution	For use in
1	<i>E. coli</i> Lysate Bottle 1	Reconstitute the lyophilizate with <b>0.19 ml</b> of Reconstitution Buffer (bottle 5), mix carefully by rolling or gentle shaking. Do not vortex!	<ul style="list-style-type: none"> <li>• section 3.3.2</li> <li>• solution 8</li> </ul>
2	Reaction Mix Bottle 2	Reconstitute the lyophilizate with 0.8 ml of Reconstitution Buffer (bottle 5), mix by rolling or shaking.	<ul style="list-style-type: none"> <li>• section 3.3.2</li> <li>• solution 8</li> </ul>
3	Feeding Mix Bottle 3	Reconstitute the lyophilizate with 10.5 ml of Reconstitution Buffer (bottle 5), mix by rolling or shaking.	<ul style="list-style-type: none"> <li>• section 3.3.2</li> <li>• solution 7</li> </ul>
4	Energy Mix Bottle 4	Reconstitute the lyophilizate with 0.6 ml of Reconstitution Buffer (bottle 5), mix by rolling or shaking.	<ul style="list-style-type: none"> <li>• section 3.3.2</li> <li>• solution 7</li> <li>• solution 8</li> </ul>
5	Reconstitution Buffer Bottle 5	<ul style="list-style-type: none"> <li>• 2 × 35 ml</li> <li>• Ready-to-use solution</li> <li>• The solution is stable at 2–8°C but can also be stored at –15 to –25°C</li> </ul>	solutions 1, 2, 3, 4

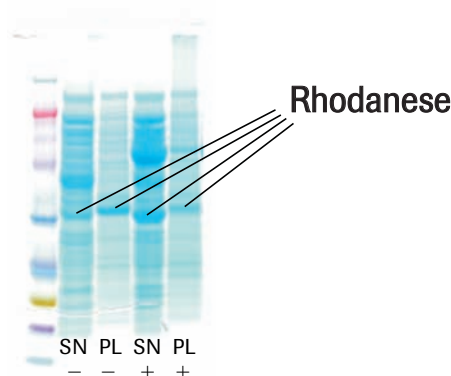
**Appearance of solutions** With the exception of the *E. coli* lysate all reconstituted lyophilizates should be clear solutions.

#### 3.3.2 Preparation of working solutions

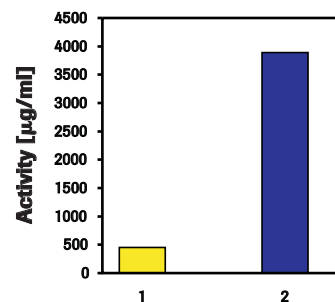
Solution	Content	Reconstitution/Preparation of working solution	For use in
7	Feeding Solution	Add 0.5 ml of the reconstituted Energy Mix (solution 4) to Feeding Mix (solution 3). Mix by rolling or shaking to obtain Feeding Solution.	section 3.1.4 of the manual of Cat. No. 03 018 008 001
8	Reaction Solution	To <i>E. coli</i> lysate (solution 1) add 0.75 ml of the reconstituted Reaction Mix (solution 2), 60 µl RTS GroE supplement, 50 µl of the reconstituted Energy Mix (solution 4) and 5–15 µg of the DNA template (50 µl maximum volume). Mix carefully by rolling or gentle shaking. DO NOT VORTEX!	section 3.1.4 of the manual of Cat. No. 03 018 008 001

**Reaction set-up** Please refer to section 3.1.4. of the manual of Cat.No. 03 018 008 001.

The protein Rhodanese (35 kD) needs the assistance of GroE to be soluble and functional. We therefore chose Rhodanese as a model protein and expressed it in the presence or absence of RTS GroE supplement using the RTS 500 *E. coli* HY Kit. The effect of the supplement on solubility and functionality was analyzed by SDS-PAGE (Fig. 1) and an enzymatic activity assay (Fig. 2).



**Fig. 1:** Coomassie-stained SDS gel of Rhodanese (35 kD) expressed in RTS 500 *E. coli* HY. SN: reaction supernatant PL: precipitated protein +: GroE supplement added -: no GroE supplement added. The ratio of soluble versus insoluble protein is significantly enhanced by GroE addition.



**Fig. 2:** Total activity of RTS-expressed Rhodanese. Column 1: Expression without GroE supplement Column 2: Expression with GroE supplement

## 5. References

- 1 See "Molecular Chaperons and Folding Catalysts", Harwood Academic Publishers, 1999 (edited by Bernd Bukau): articles by Ranson, N. A. and Clarke, A. R. (pp. 491-522) and Burston, S. G. and Saibil, H. R. (pp. 523-553).
- 2 Weber, F. et al, *Nat. Struct. Biol.* **5**, 977-985 (1998).
- 3 Ewalt, K.L. et al, *Cell* **90**, 491-500 (1997).

## 6. Ordering Information

For a complete overview, please visit and bookmark our Special Interest Site: <http://www.proteinexpression.com>

Product	Pack Size	Cat. No.
<b>Linear Template Generation by PCR</b>		
RTS <i>E. coli</i> Linear Template Generation Set, His <sub>6</sub> -tag	96 reactions	03 186 237 001
RTS <i>E. coli</i> Linear Template Generation Set, HA-tag	96 reactions	03 315 860 001
RTS <i>E. coli</i> Linear Template Generation Set, MBP fusion	96 reactions	03 358 828 001
<b>Rapid Expression Screening and Optimization</b>		
RTS 100 <i>E. coli</i> HY Kit	24 reactions 96 reactions	03 186 148 001 03186 156 001
<b>Preparative-Scale Expression</b>		
RTS 500 ProteoMaster <i>E. coli</i> HY Kit <sup>1,3,4,5</sup>	5 reactions	03 335 461 001
RTS 500 <i>E. coli</i> HY Kit <sup>2</sup>	2 reactions 5 reactions	03 246 817 001 03 246 949 001
RTS 9000 <i>E. coli</i> HY Kit	1 reaction 3 reactions	03 290 395 001 03 290 468 001
<b>AviTag Biotinylation Reagents</b>		
RTS AviTag <i>E. coli</i> Biotinylation Kit, Plasmid	For 96 reactions (RTS 100) or 5 reactions (RTS 500)	03 514 919 001
RTS AviTag Biotinylation Kit	For 96 reactions (RTS 100) or 5 reactions (RTS 500)	03 514 935 001
<b>Vectors</b>		
RTS pIVEX His <sub>6</sub> -Tag 2 <sup>nd</sup> Generation Vector Set	2 vectors, 10 µg each	03 269 019 001
RTS pIVEX HA-Tag Vector Set	2 vectors, 10 µg each	03 268 993 001
RTS pIVEX MBP Fusion Vector	1 vector, 10 µg	03 268 985 001
RTS pIVEX GST Fusion Vector	1 vector, 10 µg	03 268 969 001
<b>RTS Wheat Germ Products</b>		
RTS Wheat Germ Linear Template Generation Set, His <sub>6</sub> -tag	96 reactions	03 728 790 001
RTS pIVEX Wheat Germ His <sub>6</sub> -Tag Vector Set	10 µg each	03 728 803 001
RTS 100 Wheat Germ CECF Kit	24 reactions	03 728 811 001
<b>Other Reagents</b>		
RTS DnaK Supplement	1 set	03 784 843 001
RTS Amino Acid Sampler	for five RTS 500 reactions	03 262 154 001

<sup>1</sup> For use with the RTS ProteoMaster Instrument or the Eppendorf Thermomixer comfort.

<sup>2</sup> For use with the RTS 500 Instrument

\*available from Roche Applied Science

## Notice to Purchaser

For Research Use Only. Proteins expressed using the RTS, and data derived therefrom that would enable the expression of such proteins (collectively, "Expressed Proteins"), may be used only for the internal research of the purchaser of this system. Expressed Proteins may not be sold or transferred to any third party without the written consent of Roche Diagnostics." The purchase price of this product includes a limited, non-exclusive, non-transferable license under U.S. patents 6.168.931 and 6.337.191 and their foreign counterparts, exclusively licensed by a member of the Roche Group.

The continuous-exchange cell-free (CECF) technology applied in the RTS 500 and RTS 9000 products is exclusively licensed by a member of the Roche Group.

## Changes to Previous Version

Minor editorial changes

## Trademarks

RTS, PIVEX, PROTEOMASTER, and COMPLETE are trademarks of Roche.

AviTag is a trademark of Avidity LLC, Denver, Co.

Other brands and product names are trademarks of their respective holders.

## Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site** at:

**[www.roche-applied-science.com/support](http://www.roche-applied-science.com/support)**

To call, write, fax, or email us, visit the Roche Applied Science home page, [www.roche-applied-science.com](http://www.roche-applied-science.com), and select your home country. Country-specific contact information will be displayed. Use the Product Search function to find Pack Inserts and Material Safety Data Sheets.



# Diagnostics

Roche Diagnostics GmbH  
Roche Applied Science  
68298 Mannheim  
Germany