Rapid Translation System RTS GroE Supplement

Cat. No. 3 263 690
For five 1 ml reactions

Version 1, August 2001
Store at −15 to −25°C

1. Product overview

Product description
Solution, contains GroEL and GroES. The set includes 5 vials, 125 µl each, for use in the cell-free protein expression systems RTS 100 E.coli HY (Cat. No. 3 186 148), RTS 500 E.coli HY (Cat. No. 3 246 817 and 3 246 949) and RTS 500 E.coli Circular Template (Cat. No. 3 018 008).

Stability
Stable at -20°C. Freeze/thaw cycles up to three times do not decrease the activity.

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.

Safety information
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 (RTS) 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 cytoplasmatic chaperonins of *E. coli* (1,2). It is believed that they are involved in the *de novo* folding of 10 - 30% of all cytoplasmatic 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 Rapid Translation System 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. 3 186 148). 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. 3 246 817 and 3 246 949) or the RTS 500 E.coli Circular Template Kit (Cat. No. 3 018 008).

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	*E.coli* 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 shanking.	• 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 −20°C	solutions 1, 2, 3, 4

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

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3.1.2 Preparation of working solutions

Solution	Content	Reconstitution/ Preparation of working solution
7	Reaction Solution	Into one of the supplied reaction tubes pipet the following components: 1 12 µl *E.coli* Lysate 2 10 µl Reaction Mix 3 12 µl Amino Acids 4 1 µl Methionine 5 **6 µl of RTS GroE Supplement** 6 0.5 µg of the circular DNA or linear template in 10 µl of water or TE-buffer. • **Please note: the final volume will be slightly higher than in case of the standard RTS 100 *E.coli* HY protocol !** • A premix of solutions 1–6 without DNA is recommended for multiple parallel reactions. • Mix carefully by rolling or gentle shaking. • DO NOT VORTEX ! • Run the reaction

Reaction set-up Please refer to section 3.1.4 of the manual of Cat. No. 3 186 148.

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	*E.coli* Lysate Bottle 1, red cap	Reconstitute the lyophilizate with **0.340** ml of Reconstitution Buffer (bottle 6), mix carefully by rolling or gentle shaking. DO NOT VORTEX!	• section 3.2.2 • solution 8
2	Reaction Mix Bottle 2, green cap	Reconstitute the lyophilizate with 0.25 ml of Reconstitution Buffer (bottle 6), mix by rolling or shaking.	• section 3.2.2 • solution 8
3	Feeding Mix Bottle 3, blue cap	Reconstitute the lyophilizate with 8.1 ml of Reconstitution Buffer (bottle 6), mix by rolling or shaking.	• section 3.2.2 • solution 7
4	Amino Acid Mix without Methionine Bottle 4, brown cap	Reconstitute the lyophilizate with 3 ml of Reconstitution Buffer (bottle 6), mix by rolling.	• section 3.2.2 • solution 7 and 8
5	Methionine Bottle 5, yellow cap	Reconstitute the lyophilizate with 1.8 ml of Reconstitution Buffer (bottle 6), mix by rolling or shaking.	• section 3.2.2 • solution 7 and 8
6	Reconstitution Buffer Bottle 6, white cap	• Ready-to-use solution • The solution is stable at 2–8°C but can also be stored at −20°C	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. 3 246 817 and 3 246 949.
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). **Add 125 µl of RTS GroE supplement plus 60 µl Reconstitution buffer**. 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 DO NOT VORTEX!	section 3.1.4 of the manual of Cat. Nos. 3 246 817 and 3 246 949.

Reaction set-up Please refer to section 3.1.4. of the manual of Cat. Nos. 3 246 817 (2 reactions pack size) or 3 246 949 (5 reactions pack size).

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

3.3.1 Reconstitution of reaction components

Solution	Content	Reconstitution/Preparation of working solution	For use in
1	*E.coli* Lysate Bottle 1	Reconstitute the lyophilizate with **0.19 ml** of Reconstitution Buffer (bottle 5), mix carefully by rolling or gentle shaking. Do not vortex!	• section 3.3.2 • solution 8
2	Reaction Mix Bottle 2	Reconstitute the lyophilizate with 0.8 ml of Reconstitution Buffer (bottle 5), mix by rolling or shaking.	• section 3.3.2 • solution 8
3	Feeding Mix Bottle 3	Reconstitute the lyophilizate with 10.5 ml of Reconstitution Buffer (bottle 5), mix by rolling or shaking.	• section 3.3.2 • solution 7
4	Energy Mix Bottle 4	Reconstitute the lyophilizate with 0.6 ml of Reconstitution Buffer (bottle 5), mix by rolling or shaking.	• section 3.3.2 • solution 7 • solution 8
5	Reconstitution Buffer Bottle 5	• 2 × 35 ml • Ready-to-use solution • The solution is stable at 2–8°C but can also be stored at −20°C	solutions 1, 2, 3, 4

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

3.3.2 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. 3 018 008
8	Reaction Solution	To *E.coli* 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. 3 018 008

Reaction set-up Please refer to section 3.1.4. of the manual of Cat.No. 3 018 008

4. Example

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).

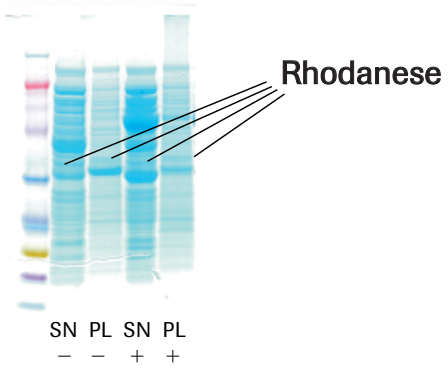

Fig. 1: Coomassie-stained SDS gel of Rhodanese (35 kD) expressed in RTS 500 *E.coli* HY.
SN: reaction supernatant
PL: precipitated protein
+: GroE supplement added
−: no GroE supplement added.
The ratio of soluble versus insoluble protein is significantly enhanced by GroE addition.

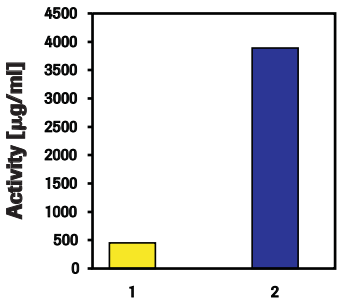

Fig. 2: Total activity of RTS-expressed Rhodanese.
Column 1: Expression without GroE supplement
Column 2: Expression with GroE supplement

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).

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7. Related products

Product	Pack Size	Cat. No.
Rapid Translation System RTS 500 Instrument	1 instrument	3 064 859
Rapid Translation System RTS 100 *E.coli* HY Kit	24 reactions (50 µl each)	3 186 148
Rapid Translation System RTS 500 *E.coli* HY Kit	2 reactions (1 ml each) 5 reactions (1 ml each)	3 246 817 3 246 949
Rapid Translation System RTS 500 *E.coli* Circular Template Kit	5 reactions (1 ml each)	3 018 008
Rapid Translation System RTS pIVEX His-tag Vector Set	5 vectors (10 µg each)	3 253 538
Rapid Translation System RTS Amino Acid Sampler	For five RTS 500 reactions	3 262 154
Restriction Protease Factor Xa	3 × 30 µg 3 × 100 µg 3 × 250 µg	1 179 888 1 585 924 1 179 896
Restriction Protease Factor Xa Cleavage and Removal Kit	Kit I: 3 × 30 µg Kit II: 3 × 100 µg	1 644 777 1 644 785
rGFP	50 µg	1 814 524
Anti-His_6	100 µg	1 922 416
Anti-His_6-Peroxidase	50 U	1 965 085
Complete Protease Inhibitor Cocktail Tablets, mini, EDTA-free	25 tablets (each tablet for 10 ml extract)	1 836 170

*available from Roche Applied Science

E-mail Address	Country
argentina.biochem@roche.com	Argentina
biochem.au@roche.com	Australia
Gerhard.Muehlbauer@roche.com	Austria
biochem.be@roche.com	Belgium
Valent@mbox.cit.bg	Bulgaria
africhem@camnet.cm	Cameroon
biochem.ca@roche.com	Canada
biochem.cn@roche.com	China
Info@medisell.com.cy	Cyprus
dalimil.zurek@roche.com	Czech Republic
dk.biochem@roche.com	Denmark
surgitech@surgitech.ee	Estonia
pharsc.et@telecom.net.et	Ethiopia
helsinki.biochem_diagnostics@roche.com	Finland
biochem.fr@roche.com	France
mannheim.biocheminfo@roche.com	Germany
Bm_roche@hotmail.com	India
h.hajian@tebtech.com	Iran
tubanegin@istn.irost.com	Iran
Dyn@netvision.net.il	Israel
it.biochem@roche.com	Italy
biochemicals@rdj.co.jp	Japan
pharmakp@net2000ke.com	Kenya
Bmskorea@chollian.net	Korea
react@ncc.moc.kw	Kuwait

E-mail Address	Country
Raitis@invitros.lv	Latvia
Sakkijha@rdleb.com	Lebanon
Gintaras@eksma.lt	Lithuania
diagnostics@prophac.lu	Luxembourg
Vccl@vol.net.mt	Malta
Aiouche.echo@dounia.net.ma	Morocco
almere.as@roche.com	Netherlands
biochem.nz@roche.com	New Zealand
bofungwu@linkserve.com.ng	Nigeria
biochem.se@roche.com	Norway
biochem.pt@roche.com	Portugal
Topdiag@fx.ro	Romania
biochem.sg@roche.com	Singapore
roche.diagnostics@siol.net	Slovenia
south_africa.bioboffin@roche.com	South Africa
biochem.es@roche.com	Spain
biochem.se@roche.com	Sweden
BiochemInfo.CH@roche.com	Switzerland
Jean-Marie.kindbeiter@roche.com	Tunisia
taiwan.diagnostics@roche.com	Taiwan
bmuae@emirates.net.ae	United Arab Emirates
uk.biochem@roche.com	United Kingdom
biochemts.us@roche.com	USA
Mvalentiner@telcel.net.ve	Venezuela
dusica@eunet.yu	Yugoslavia
biochemts.row@roche.com	All other countries

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Argentina 541 954 5555; **Australia** (02) 9899 7999; **Austria** (01) 277 87; **Belgium** (02) 247 4930; **Brazil** +55 (11) 3666 3565; **Bulgaria** +35929625408; **Cameroon** 237-370269; **Canada** (450) 086 7050; (800) 361 2070; **Chile** 00 56 (2) 22 33 737 (central) 00 56 (2) 22 32 099 (Exec); **China** 86 21 6427 5586; **Colombia** 0057-1-3412797; **Cyprus** +357-2-311362; **Czech Republic** (02) 20382 564-5; **Denmark** +45 363 999 58; **Egypt** 20-2-3619047; **Estonia** +372 626 1305; **Ethiopia** 251-1-552799; **Finland** +358 9 525 333 66; **France** 04 76 76 30 87; **Germany** (0621) 759 8568; **Greece** +3 01 61 66 100; **Hong Kong** (852) 2485 7596; **India** +91-22-8379906; **Indonesia** 62 (021) 252 3820 ext. 755; **Iran** +98-21-8072374 / +98-21-8797027; **Israel** 972-6- 6380569; **Italy** 039 247 4109-4181; **Japan** 03 5443 5284; **Kenya** +254-2-750112; **Korea** 82-2-3471-6500; **Kuwait** +965-4837859; **Latvia** 371-787828309; **Lebanon** Fax: 00961-1-399667; **Lithuania** 370-2-729715; **Luxembourg** +352-496098; **Malta** Fax: +356-341087; **Morocco** Fax: +212-2-944040; **Malaysia** 60 (03) 755 5039; **Mexico** (5) 227 8967; **Netherlands** (036) 539 4911; **New Zealand** (09) 276 4157; **Nigeria** +234-1-521767; **Norway** (47) 23 373300; **Philippines** (632) 810 7246; **Poland** +48 (22) 22 66 84 305; **Portugal** (01) 4171717; **Republic of Ireland** 1 800 40 90 41; **Romania** +40-1-2123763; **Russia** (49) 621 759 8636 Fax: (49) 621 759 8611; **Saudia Arabia** +966-1-4010364; **Singapore** 0065 272 9200; **Slovenia** +386 61 1363528; **South Africa** (011) 886 2400; **South Korea** 02 569 6902; **Spain** (93) 201 4411; **Sweden** (08) 404 8800; **Switzerland** +41 (41) 799 6160; **Taiwan** (02) 25052533; **Thailand** 66 (2) 274 07 08 (12 line); **Turkey** 0090 212 216 32 80; **United Arab Emirates** +971-4-694351; **United Kingdom** (0800) 521578; **USA** (800) 428 5433. **Venezuela** Fax: +0058-4810697; **Yugoslavia** +381 11 137163.

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

Roche Diagnostics GmbH
Roche Applied Science
Sandhofer Strasse 116
D-68305 Mannheim
Germany