*Roche Applied Science*
*Rapid Translation System*
*Application Note No. 10/2002*

RTS

# Detergents in RTS

## Introduction

Proteins synthesized *in vivo* as well as *in vitro* are often obtained as insoluble aggregates, making protein characterization difficult. With the open design of the RTS it is now possible to check the influence of detergents, amphipathic molecules that can interact with hydrophobic parts of proteins and thus prevent the aggregation during the translation reaction. It has been observed that the refolding process of denatured proteins (e.g. by urea) can be enhanced in the presence of detergents (Zardeneta & Horowitz, 1992; Rozema & Gellman, 1996).
Since the proteins are in a non-native state during and after translation the situation should, in principle, be comparable to the refolding of proteins from a denatured state through partly folded intermediates, which are still prone to aggregation.
In this state, the detergent molecule can capture the exposed hydrophobic regions of the protein, which would otherwise lead to irreversible aggregation. Thus the synthesized protein can be kept in a folding competent state until the folding machinery of the lysate fulfils its task. This beneficial property of detergent molecules was analyzed utilizing the RTS 100 *E. coli* HY kit, where rapid optimization can be performed, and subsequently scaled up using the RTS 500 *E. coli* HY kit.
The advantages of RTS over an *in vivo* expression system made it possible to analyze the effects of various detergents in different concentrations on the efficiency of the *in vitro*-system to synthesize proteins in a soluble form.

# Compatibility of different detergents with RTS HY biochemistry

To test the compatibility of detergents with the RTS 100 *E. coli* HY and RTS 500 *E. coli* HY, two proteins were expressed in presence of different detergents. The two proteins were chloramphenicol acetyltransferase (CAT) and a fusion protein of GFP with a dimer of the anti-bacterial peptide CecropinP1 (GFP(CecropinP1)$_2$), both as C-terminally tagged constructs (CAT: His$_6$-tag, GFP(CecropinP1)$_2$: Strep-tag). 18 detergents were used in the concentrations below, at and above the corresponding critical micellar concentration (CMC) at 30°C (Table 1).
Below the CMC, none of the detergents tested showed a harmful effect on the expression of the test protein CAT. Most of the detergents could also be added at their CMCs without any major inhibition of the reaction. At concentrations higher than the CMC, three of the detergents (all three of them non-ionic) with a defined CMC showed no negative effects. The majority of the tested detergents were incompatible with RTS at higher concentrations.

**Table 1:** General overview of detergents that were tested in the RTS 100 *E. coli* HY and the RTS 500 *E. coli* HY format: positive/negative influence

| Detergent | M.W. | CMC (mM) | <CMC | =CMC | >CMC |
|---|---|---|---|---|---|
| **a) ionic detergents** | | | | | |
| Desoxycholic acid | 414.6 | 2 – 6 | x | — | — |
| **b) non-ionic** | | | | | |
| APO – 10 [1)] | 218.3 | 4.6 | x | — | — |
| Brij 35 | 1199.6 | 0.09 | x | x | — |
| Brij 58P | 1122 | 0.077 | x | x | x |
| Decyl-ß-D-maltopyranoside | 482.6 | 1.6 | x | x | — |
| n-Dodecyl-ß-D-maltoside | 510.6 | 0.1 – 0.6 | n.d. | x | — |
| Mega – 8 [1)] | 321.5 | 58 | x | — | — |
| Mega – 10 | 349.5 | 6 – 7 | x | x | — |
| NP – 40 | 603.0 | 0.05 – 0.3 | x | x | x |
| Triton X-100 | 625 | 0.2 – 0.9 | x | x | x |
| Triton X-114 | 558.75 | 0.35 | x | x | — |
| Tween20 | 1228 | 0.06 | x | x | — |
| **c) zwitterionic** | | | | | |
| Chaps | 614.9 | 6 – 10 | x | x | — |
| Zwittergent 3 – 12 | 335.6 | 2 – 4 | x | — | — |
| Zwittergent 3 – 14 | 363.6 | 0.1 – 0.4 | x | — | — |

x : suitable for use in RTS *E. coli* HY reactions
— : the expression yield in the RTS 500 *E. coli* HY format decreases over 20 %
1): only tested in RTS 500 *E. coli* HY reactions

# Examples:

## 1. Influence of different detergents on the expression of CAT:

The following table shows the concentration range of the detergents used in the RTS 500 *E. coli* HY and the RTS 100 *E. coli* HY kit and the specific effects on the solubility and expression yield. Several detergents like Deoxycholic acid or Tween 20 had no effect on solubility and expression yield of Chloramphenicol acetyltransferase (CAT).
The reactions were run according to the pack insert. For the determination of solubility, the synthesized protein was quantified via Coomassie-stained SDS-PAGE. Pellets and supernatants were analyzed after a centrifugation step of 10 minutes at 14000 rpm.

**Table 2:** Influence of detergents at various concentrations on the expression of CAT

| Detergent | CMC (mM) | conc. (mM) | solubility | expression yield |
|---|---|---|---|---|
| **a) non-ionic** | | | | |
| APO – 10 | 4.6 | 1.0<br>4.6 – 10 | o<br>— — | o<br>— — |
| Brij 35 | 0.09 | 0.09 – 1.0 | o | o |
| | | 10 – 50 | — | — |
| Brij 58P | 0.077 | 0.5 – 3.0 | o | o |
| NP – 40 | 0.05 – 0.3 | 0.05 – 0.3<br>2 – 5 | o<br>— | o<br>+ |
| Triton X-100 | 0.2 – 0.9 | 0.05 – 0.1 | + | o |
| | | 0.2 | + | + |
| | | 1.5 | — | o |
| **b) zwitterionic** | | | | |
| Chaps | 6 – 10 | 1<br>6 – 10<br>50 – 80 | o<br>+<br>— | o<br>o<br>— |
| Zwittergent 3 – 12 | 2 – 4 | 0.5<br>2<br>4 – 10 | +<br>— —<br>— — | o<br>—<br>— — |
| Zwittergent 3 – 14 | 0.1 – 0.4 | 0.1<br>0.4<br>1.5 – 10 | o<br>— —<br>— — | +<br>—<br>— — |
| **d) non-detergent sulfobetaine** | | | | |
| NDSB 195 | — | 5 – 10<br>50<br>100 | o<br>+<br>o | o<br>o<br>o |
| NDSB 201 | — | 5 – 10<br>50<br>100 | o<br>+<br>o | o<br>o<br>o |

Yields were compared to controls expressed without the additions of detergents.
o : no negative effects when detergents were added
+ : increase of the expression yield in the 500 *E. coli* HY-system greater than 20 %
– : decrease of the expression yield in the 500 *E. coli* HY-system greater than 20 %
– – : decrease of the expression yield in the 500 *E. coli* HY-system greater than 50 %

## 2. Soluble expression of GFP(CecropinP1)$_2$:

CecropinP is an antibacterial peptide, isolated from the porcine intestine [Vunnam et al., 1997]. Expression of the fusion protein GFP(CecropinP1)$_2$ in RTS 100 *E. coli* HY and RTS 500 *E. coli* HY resulted in insoluble aggregates. The result of testing different detergents in the RTS showed that Brij 58 P (Figure 1) and Brij 35 (data not shown) could enhance the solubility of GFP (CecropinP1)$_2$, without negative effect on the yield.
The following figure shows the effects of the detergent Brij 58P in the RTS 100 *E. coli* HY and the RTS 500 *E. coli* HY system.

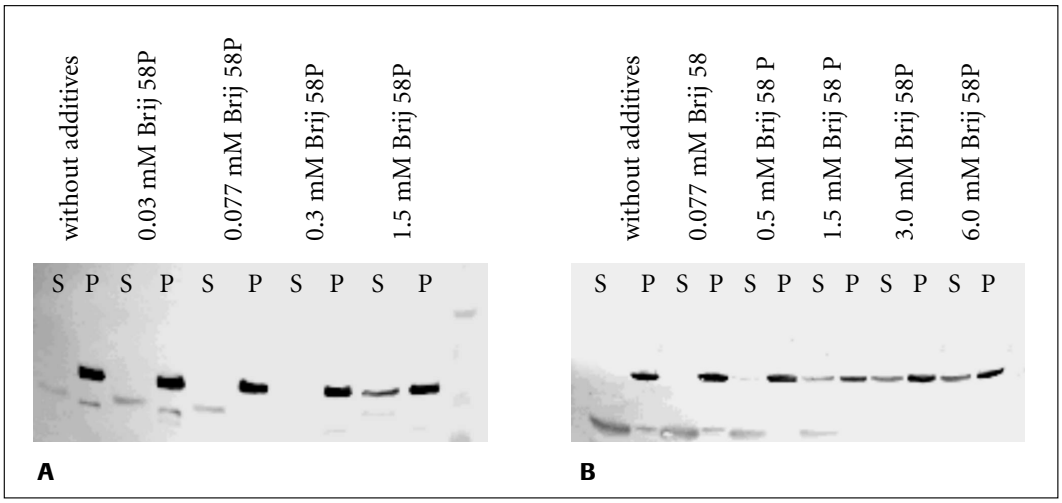


**Figure 1:** Westernblot analysis of the protein GFP (CecropinP1)$_2$ after expression in RTS 100 *E. coli* HY (**A**) and RTS 500 *E. coli* HY (**B**); S = supernatant, P = pellet

These data indicate that detergents can be a useful tool to improve the protein expression in RTS, since they can have positive effects on solubility and/or the expression yield. The optimal concentration of the detergent supplement can easily be found in RTS 100 *E. coli* HY and subsequently scaled up to the RTS 500 *E. coli* HY format.

## Protocol for addition of detergents in the RTS 500 *E. coli* HY Kit:

The design of the kit allows the addition of up to 200 µl of supplement solution to the reaction solution and/or 2ml to the feeding solution.

**1. Reconstitution of reaction components:**

| | |
|---|---|
| *E. coli* Lysate<br>Bottle 1, red cap | Reconstitute the lyophilizate with **0.340** ml of reconstitution buffer, mix carefully by rolling or gentle shaking |
| Reaction Mix<br>Bottle 2, green cap | Reconstitute the lyophilizate with **0.25** ml of reconstitution buffer, mix by rolling or shaking |
| Feeding Mix<br>Bottle 3, blue cap | Reconstitute the lyophilizate with **6.1** ml of reconstitution buffer, mix by rolling or shaking |
| Amino Acid Mix<br>without Methionine<br>Bottle 4, brown cap | Reconstitute the lyophilizate with **3** ml of reconstitution buffer, mix by rolling |
| Methionine<br>Bottle 5, yellow cap | Reconstitute the lyophilizate with **1.8** ml of reconstitution buffer, mix by rolling or shaking |
| Detergents | Make a stock solution of the required detergents |

**2. Preparation of working solutions**

| Content | Reconstitution/Preparation of working solution |
|---|---|
| Feeding solution | Add **2.65** ml of the reconstituted Amino Acid Mix without Methionine and **0.3** ml of reconstituted Methionine to solution 3.<br>Finally add up to **2** ml of the detergent stock solution (depending on the concentration of the stock solution). If less than **2** ml stock solution have been added, make the total volume up to **11** ml with reconstitution buffer . |
| Reaction solution | To the content of solution 1 add **0.225** ml of the reconstituted reaction Mix, **0.27** ml of the reconstituted Amino Acid Mix without Methionine and **30** µl of reconstituted Methionine. Add **15** µg of DNA template in a maximum volume of 50 µl. Finally add up to **200** µl of the stock solution (depending on the concentration of the stock solution). If less than **200** µl stock solution have been added, make the total volume up to **1.1** ml with reconstitution buffer. |

(Instruction Manual, Version 1, June 2001: "Rapid Translation System RTS 500 *E. coli* HY Kit", Roche Applied Science)

**Example with the detergent Brij 58P:**
e. g. Stock solution: 10 mM (in RNase free water or reconstitution buffer)
e. g. Final concentration: 1.5 mM
**Feeding solution:**
To the prepared reconstituted feeding solution, add 1.65 ml stock solution Brij 58P (10 mM) and add 0.35 ml reconstitution buffer.
**Reaction solution:**
To the prepared reconstituted reaction solution, add 165 µl stock solution Brij 58 P (10 mM) and add 35 µl reconstitution buffer.

## References


1) Rozema, D. and Gellman, S.H. (1996): Artificial chaperone-assisted refolding of carbonic anhydrase B, *Journal of Biological Chemistry*, **271** (7), 3478-3487.
2) Zardenata, G. and Horowitz, P.M. (1992): Micelle-assisted protein folding, *Journal of Biological Chemistry*, **267** (9), 5811-5816.
3) Vunnam, S., Juvvadi, P., Merrifield, R.B. (1997): Synthesis and antibacterial action of cecropin and proline-arginine-rich peptides from pig intestine, *Journal of Peptide Research*, **49** (1), 59-66.





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