

DS supplement

#PF005-0.5-EX

For 500 µL Reaction

PURE_{frex}® 2.1 (#PF213) is NOT included.

Lot : _____

Expiry Date : _____

in vitro research use only

Store at -80°C before opening

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Introduction

1. About PURE_{frex}®

PURE_{frex}® is a reconstituted cell-free protein synthesis kit which GeneFrontier has developed based on the PURE system technology. The target protein can be synthesized by adding the template DNA (or mRNA) to the reaction mixture. The PURE system is a unique cell-free protein synthesis system, which has originally developed by Professor Takuya Ueda at the University of Tokyo, and consists of only purified factors necessary for transcription, translation and energy regeneration (Ref. 1). Therefore it enables to adjust the composition of the reaction mixture.

PURE_{frex}® has been raised in purity by improving the methods for preparing ribosomes, tRNAs and all proteins in the reaction mixture compared with the original PURE system (Ref. 2). As the result, the contaminating lipopolysaccharide from *E. coli* is reduced to around 0.1 EU per 1 µL of reaction and other contaminants, such as RNase and β-galactosidase, are also reduced.

Because all of proteins in PURE_{frex}® have no tags, the synthesized protein can be purified and detected by any tags.

References 1) Shimizu *et al.* (2001) *Nat. Biotechnol.*, vol. 19, p. 751

2) Shimizu *et al.* (2005) *Methods*, vol. 36, p. 299

Introduction

2. About DS supplement

Formation of disulfide bonds is an important process for folding and stability of most of secretory proteins such as enzyme or antibody. Because a disulfide bond is usually formed by the oxidation of sulfhydryl groups (-SH) of adjacent cysteine residues, oxidative environment is necessary to form a disulfide bond.

Additionally, disulfide bond isomerase which can catalyze the disulfide bridge exchange may be also required for a correct pairing of cysteines.

DS supplement includes oxidized glutathione (GSSG) and DsbC protein (disulfide bond isomerase in *E. coli*). Addition of DS supplement to PURE_{frex}® enables a protein containing disulfide bonds to be synthesized in an active form.

Efficiency of the formation of disulfide bonds is dependent on reductant in the reaction mixture. The suitable reductant can be selected by using PURE_{frex}® 2.1 (#PF213).

DnaK Mix (#PF003), molecular chaperone, also works very well with DS supplement in a same tube for protein synthesis reaction, which could lead to the preparation of your protein in proper form with good activity.

Note

DS supplement is developed for *in vitro* research use only. DS supplement should not be used for the therapy, diagnostic or administration to animals including human and should not be used as food or cosmetics etc.

To avoid the contamination of nuclease, nuclease-free-treated water, reagents and materials should be used. We also recommend wearing gloves and mask.

For information concerning commercial use of DS supplement, please contact GeneFrontier.

e-mail : purefrex@genefrontier.com



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Distributor



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Kit components

GSSG (Green)^{*1} 25 µL

Oxidized glutathione (60 mM)

Store at -20°C

DsbC (Green)^{*2} 25 µL

E. coli DsbC (7.5 mg/mL)

Store at -80°C^{*3}

Dilution Buffer (Clear) 500 µL

30% glycerol buffer

Store at -20°C

Kit components

Store at -80°C before opening

^{*1)}

Standard final concentration of GSSG is 3 mM.

We recommend the optimizing the concentration of any reducing agent to achieve higher activity because it is depends on the synthesized protein.

^{*2)}

Standard final concentration of DsbC is 23 - 375 µg/mL.

We recommend the optimizing the concentration of any reducing agent to achieve higher activity because it is depends on the synthesized protein.

Please use attached Dilution Buffer for dilution of DsbC.

^{*3)}

For storage at -80°C, the rest of solution should be frozen rapidly in liquid nitrogen or dry ice/ethanol. Divide into aliquots, if necessary, and avoid refreeze and thaw as much as possible.

Protocol

This is a standard protocol for synthesizing proteins containing disulfide bonds. Each solution of DS supplement and PURE_{frex}® 2.1 (#PF213) are mixed together in a same tube. For example, 20 µL of reaction is assembled as below, which final concentration of each reagent will be 0.5 mM Cysteine, 4 mM GSH and 3 mM GSSG and 94 µg/mL DsbC.

1. Thaw Solution I, Cysteine, GSH and GSSG by incubation at room temperature or 37 °C for 1 minute completely, and then cool on ice.
2. Thaw Solution II, III and DsbC on ice.
3. Mix each solution by vortex and centrifuge briefly to collect each solution at the bottom.
4. Assemble the reaction mixture in a tube as follows.
(Add the template DNA to 1-3 ng/µL per 1 kbp)

Protocol

| | |
|--------------------------------|--------|
| Water | 5-X µL |
| Solution I ^{*4} | 8 µL |
| 10 mM Cysteine | 1 µL |
| 80 mM GSH | 1 µL |
| 60 mM GSSG | 1 µL |
| Solution II | 1 µL |
| Solution III | 2 µL |
| 1.875 mg/mL DsbC ^{*5} | 1 µL |
| Template DNA | X µL |
| Total | 20 µL |

5. Incubate the tube at 37°C for 2-6 hours.

Protein synthesis reaction is almost done until 6 hours, but some proteins require longer incubation (e.g. 24 hours) to form disulfide bonds between the correct pair of cysteine residues.

6. Analyze the synthesized product.

^{*4)}

Please note that the volume of Solution I of PURE_{frex}® 2.1 (#PF213) is different from PURE_{frex}® 2.0 (#PF201).

^{*5)}

Please use the attached Dilution Buffer to dilute DsbC.