DsbC Set

Former **DS supplement**

#PF005-0.5-EX

For 500 µL Reaction

PURE*frex*® is NOT included.

in vitro research use only

Store at -80ºC 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. Biotecnol.*, vol. 19, p. 751
2. Shimizu *et al.* (2005) *Methods*, vol. 36, p. 299

Introduction

2. About DsbC Set / PDI Set

Formation of a disulfide bond is one of an important process for folding and stability of most of secretory proteins such as enzymes or antibodies.

Oxidative environment is necessary to form a disulfide bond because a disulfide bond is formed by the oxidation of sulfhydryl groups (-SH) of adjacent cysteine residues. Disulfide bond isomerase, which can catalyze the exchange of disulfide bridges, may be also required for a correct pairing of cysteines.

DsbC Set (#PF005) includes oxidized glutathione (GSSG) and *E. coli* DsbC protein (disulfide bond isomerase in the periplasm of *E. coli*).

PDI Set (#PF006) includes oxidized glutathione (GSSG), human PDI (protein disulfide isomerase) and human Ero1 α (ER oxidoreductin-1 to reoxidize PDI).

Addition of DsbC Set or PDI Set 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 reducing agent in the reaction mixture. We recommend the use of PURE*frex*® *2.1* (#PF213) in which the suitable reducing agent can be selected.

Note

DsbC Set 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.

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Kit components

GSSG (Green) [*1]	**25**	**µL**
Oxidized glutathione (60 mM)		
	Store at -20ºC	
DsbC (Green) [*2]	**25**	**µL**
E.coli DsbC (320 µM)		
	Store at -80ºC [*3]	
Dilution Buffer (Clear)	**500**	**µL**
30% glycerol buffer		
	Store at -20ºC	

Kit components

Store at -80 ºC 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)
The concentration unit has been changed from "mg / mL" to "µM" . 7.5mg / mL is equal to 320µM.
Standard final concentration of DsbC is 1 - 16 µM.
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 -80ºC, 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 DsbC Set 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 4 µM DsbC.

(Materials)
PURE*frex*® *2.1* (#PF213) contains Solution I, Solution II, Solution III, Cysteins and GSH.
DsbC Set (#PF005) contains DsbC and GSSG.

1. Thaw Solution I, Cysteine, GSH and GSSG by incubation at room temperature or 37 ºC 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. Dilute DsbC 4-fold with Dilution Buffer.
5. 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
80 mM DsbC [*5]	1 µL
Template DNA	X µL
Total	20 µL

6. Incubate the tube at 37ºC 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.
7. 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 4-fold diluted DsbC.