

DsbC Set

#PF005-0.5-EX For 500 µL Reaction

(PUREfrefx[®] is NOT included.)

In vitro research use only
Store at -80°C before opening

Introduction

1. 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 PUREfrefx[®] 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 PUREfrefx[®] 2.1 (#PF213) in which the suitable reducing agent can be selected.

2. About PUREfrefx[®]

PUREfrefx[®] 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.

PUREfrefx[®] 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 less than 1 EU per 1 µL of reaction and other contaminants, such as RNase and β -galactosidase, are also reduced.

Because all of proteins in PUREfrefx[®] 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

Kit components

	Volume	0.5		
• GSSG (Green)	25 µL	×1		
	Oxidized glutathione (60 mM) Store at -20°C			
• DsbC (Green) *1	25 µL	×1		
	<i>E. coli</i> DsbC (320 µM) Store at -80°C *2			
• Dilution Buffer (Clear)	500 µL	×1		
	30% glycerol buffer Store at -20°C			

Store at -80°C before opening

- *1) 320 µM in DsbC Set is the same concentration as 7.5 mg/mL in the former DS Supplement.
*2) For storage at -80°C, the remaining solution should be frozen rapidly in liquid nitrogen or a freezing mixture of dry ice and ethanol. Please 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 PUREfrefx[®] 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.

1. Thaw completely Solution I, Cysteine, GSH and GSSG by incubation at room temperature or 37°C for 5 minutes for completely dissolving, and then leave at room temperature.
2. Thaw Solution II, III and DsbC on ice.
3. Mix each solution by vortex and centrifuge briefly to collect 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 0.5-3 ng/µL per 1 kbp)

Water	5-X	µL
Solution I	8	µL *3
10 mM Cysteine	1	µL
80 mM GSH	1	µL
60 mM GSSG	1	µL *4
Solution II	1	µL
Solution III	2	µL
80 µM DsbC	1	µL *5
Template DNA	X	µL
Total	20	µL

6. Incubate the tube at 37°C for 4-6 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.

*3) Please note that the volume of Solution I in PUREfrefx[®] 2.1 (#PF213) is different from PUREfrefx[®] 2.0 (#PF201).

*4) The standard concentration of GSSG is 1-3 mM. We recommend to check the optimal concentration of GSSG because it depends on the protein of interest and the kind and concentration of reducing agent.

*5) The standard concentration of DsbC is 1-16 µM. The optimal concentration of DsbC depends on the protein of interest. Please use Dilution Buffer for dilution of DsbC.

Note

DsbC Set is developed for *in vitro* research use only. DsbC Set 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 any commercial use of DsbC Set, please contact GeneFrontier in advance.

e-mail : purefrefx@genefrontier.com

Distributor



COSSMO BIO USA
[Outside Japan]
2792 Loker Ave West, Suite 101, Carlsbad, CA 92010, USA
email: info@cosmobiousa.com
Phone/FAX: (+1) 760-431-4600
URL: www.cosmobiousa.com



COSSMO BIO CO., LTD.

[JAPAN]
TOYO EKIMAE BLDG. 2-20, TOYO 2-CHOME,
KOTO-KU. TOKYO 135-0016, JAPAN
Phone: +81-3-5632-9610 FAX: +81-3-5632-9619
URL: https://www.cosmobio.co.jp/

Manufacturer



GeneFrontier
www.genefrontier.com

GeneFrontier Corporation
SHARP Kashiwa Building, 4F, 273-1 Kashiwa,
Kashiwa-shi, Chiba 277-0005 Japan