PDI Set

#PF006-10ML-EX For 2 mL x 5 Reaction
#PF006-50ML-EX For 2 mL x 25 Reaction

(PURE*frex*® is NOT included.)

In vitro research use only
Store at -80ºC 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 Ero1α (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.

2. 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 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 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

Kit components

	Volume	10ML	50ML	
• **GSSG (Green)**	**100 µL**	×5	×25	
	Oxidized glutathione (60 mM) Store at -20ºC			
• **PDI (Green)**	**100 µL**	×5	×25	
	Human protein disulfide isomerase (200 µM) Store at -80ºC *1			
• **Ero1α (Green)**	**100 µL**	×5	×25	
	Human ER oxidoreductin-1 (5 µM) Store at -80ºC *1			
• **Dilution Buffer (Clear)**	**500 µL**	×1	×1	
	30% glycerol buffer Store at -20ºC			

Store at -80ºC before opening

*1) For storage at -80ºC, 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 PDI 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 1 mM GSSG and 10 µM PDI.

Ero1α can be also used instead of GSSG. Standard molar concentration ratio of PDI:Ero1α is 40:1. For example, add 0.25 µM Ero1α with 10 µM PDI to the reaction mixture. *2

1. Thaw completely Solution I, Cysteine, GSH and GSSG by incubation at room temperature or 37ºC for 5 minutes for completely dissolving, and then leave at room temperature.
2. Thaw Solution II, III and PDI on ice.
3. Mix each solution by vortex and centrifuge briefly to collect solution at the bottom.
4. Dilute GSSG 3-fold with water.
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
20 mM GSSG	1	µL *4
Solution II	1	µL
Solution III	2	µL
PDI	1	µL *2
Template DNA	X	µL
Total	20	µL

6. Incubate the tube at 37ºC 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.

*2) The standard concentration of PDI is 1-10 µM, and the standard concentration of Ero1α is 0.025-0.25 µM. The optimal concentration of PDI and Ero1α depends on the protein of interest. Please use attached Dilution Buffer for diluting PDI and Ero1α.

*3) Please note that the volume of Solution I in PURE*frex*® *2.1* (#PF213) is different from PURE*frex*® *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.

Note

PDI Set is developed for *in vitro* research use only. PDI 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 PDI Set, please contact GeneFrontier in advance.
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