PDI Set

#PF006-0.5-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

PDI Set and PURE*frex*® is developed for *in vitro* research use only. PDI Set and PURE*frex*® 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 PDI Set, please contact GeneFrontier.

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Kit components

GSSG	*1	**25 µL**
Oxidized glutathione (60 mM)		Store at lower than -20ºC
PDI	*2	**25 µL**
Human protein disulfide isomerase (200 µM)		Store at -80ºC *3
Ero1α	*2	**25 µL**
Human ER oxidoreductin-1 (5 µM)		Store at -80ºC *3
Dilution Buffer		**500 µL**
		Store at lower than -20ºC

Kit components

Store at -80 ºC before opening

*1)
Standard final concentration of GSSG is 1 – 3 mM with 4 mM GSH (reduced glutathione) in PUREfrex 2.1 (#PF213).
We recommend to check the optimal concentration of GSSG because it depends on the target protein and the kind and concentration of reducing agent.

*2)
Standard final concentration of PDI/Ero1α is 1/0.025 - 10/0.25 µM.
We recommend to check the optimal concentration of PDI and Ero1α because it depends on the target protein.
Please use attached Dilution Buffer for diluting PDI and Ero1α.

*3)
For storage at -80ºC, the rest of solution should be frozen rapidly in liquid nitrogen or dry ice/ethanol. Please divide into aliquots, if necessary, and avoid refreeze and thaw as much as possible.

Protocol

Here is a standard protocol for synthesizing proteins containing disulfide bonds using PDI Set and PURE*frex*® *2.1* (#PF213). For example, please assemble 20 µL of reaction mixture as below, in which the final concentration of each reagent is 0.5 mM Cysteine, 4 mM GSH, 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.

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 PDI on ice.
3. Mix each solution by vortex and centrifuge briefly to collect each 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 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
20 mM GSSG *5	1 µL
Solution II	1 µL
Solution III	2 µL
PDI	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 3-fold diluted GSSG.