Mar 2025

EF-P

#PFS052-0.5-EX For 500 µL Reaction

(PURE*frex*® is NOT included.)

In vitro research use only
Store at -80ºC before opening

Introduction

1. About EF-P

EF-P (elongation factor P) is one of the translation factors in *Escherichia coli* (*E. coli*) and a homolog of eukaryotic initiation factor 5A (eIF5A). EF-P improves the synthesis of protein containing consecutive proline residues such as Pro-Pro-Pro and Pro-Pro-Gly by promoting the formation of peptide bonds. Lysine at 34th of EF-P is post-translationally modified to β-lysillysine and the modification is important for the activity.

Because PURE*frex*® *1.0*, PURE*frex*® *2.0* and PURE*frex*® *2.1* don't contain EF-P, the synthesis efficiency of some proteins containing consecutive proline residues are low. Addition of EF-P to the PURE*frex*® reaction mixture increase the productivity of such proteins.

EF-P (#PFS052) is a supplement for PURE*frex*® that contains recombinant post-translationally modified EF-P isolated from *E. coli*.

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	0.5		
• EF-P (Clear)	**12.5 µL**	×1		
	40 µM EF-P (in 30% glycerol buffer) Store at -80ºC *1			
• Dilution Buffer (Clear)	**500 µL**	×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

Here is a standard protocol for synthesizing proteins using EF-P and PURE*frex*® *2.1* (#PF213). 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 µM EF-P.

1. Thaw completely Solution I, Cysteine, GSH 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 EF-P on ice.
3. Mix each solution by vortex and centrifuge briefly to collect solution at the bottom.
4. Assemble the reaction mixture in a tube as follows.
(Add the template DNA to 0.5-3 ng/µL per 1 kbp)

Water	6.5-X	µL
Solution I	8	µL *2
Cysteine	1	µL
GSH	1	µL
Solution II	1	µL
Solution III	2	µL
EF-P (40 µM)	0.5	µL *3
Template DNA	X	µL
Total	20	µL

5. Incubate the tube at 37ºC for 4-6 hours.
6. Analyze the synthesized product.

*2) Please note that the volume of Solution I in PURE*frex*® *2.1* (#PF213) is different from PURE*frex*® *2.0* (#PF201).

*3) The standard concentration of EF-P is 0.1-2 µM. The optimal concentration of EF-P depends on the protein of interest. Please use Dilution Buffer for dilution of EF-P.

Note

EF-P is developed for *in vitro* research use only. EF-P 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 EF-P, please contact GeneFrontier in advance.
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