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
Feb 2023

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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.

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 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 (#PFS052-0.5) is a supplement for PURE*frex*® that contains recombinant post-translationally modified EF-P isolated from *E. coli*.

Note

EF-P and PURE*frex*® is developed for *in vitro* research use only. EF-P 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 EF-P, please contact GeneFrontier.

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Kit components

- **EF-P** *1 — **12.5 µL**
  40 µM EF-P (in 30 % glycerol buffer)
  Store at -80ºC*2
- **Dilution Buffer** — **500 µL**
  Store at lower than -20ºC

Kit components

Store at -80 ºC before opening

*1)
Standard final concentration of EF-P is 0.1 – 2 µM.
We recommend to check the optimal concentration of EF-P because it depends on a protein of interest.

*2)
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 using EF-P 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 and 1 µM EF-P.

1. Thaw Solution I, Cysteine and GSH by incubation at room temperature or 37 ºC for 1 minute completely, and then cool on ice.
2. Thaw Solution II, III and EF-P on ice.
3. Mix each solution by vortex and centrifuge briefly to collect each 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 *3	8 µL
10 mM Cysteine	1 µL
80 mM GSH	1 µL
Solution II	1 µL
Solution III	2 µL
40 µM EF-P	0.5 µL
Template DNA	X µL
Total	20 µL

Protocol

5. Incubate the tube at 37ºC for 2-6 hours with heat block or water bath.
6. Analyze the synthesized product.

*3)
Please note that the volume of Solution I of PURE*frex*® *2.1* (#PF213) is different from PURE*frex*® *2.0* (#PF201).