Reconstituted cell-free protein synthesis kit

PURE*frex*® 2.1

#PF213-0.25-5-EX

For 5x 250 µL Reaction

in vitro research use only

Store at -80ºC before opening

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Introduction

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.

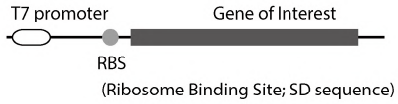
PURE*frex*® has been raised in purity by improving the methods for preparing ribosomes, tRNAs and all proteins in the reaction mixture compared with the original PURE system (Ref. 2). As the result, the contaminating lipopolysaccharide from *E. coli* is reduced to around 0.1 EU per 1 µL of reaction and 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. Biotechnol.*, vol. 19, p. 751.
2. Shimizu *et al.* (2005) *Methods*, vol. 36, p. 299.

Template DNA

1. Construction of the template DNA



The template DNA for the protein synthesis by PURE*frex*® should contain T7 promoter and ribosome binding site (SD sequence) at the upstream of the gene encoding the target protein.

At the downstream of stop codon, more than 10 nucleotides are required.

All three stop codons are available.

Both circular and linear DNAs are available as a template DNA.

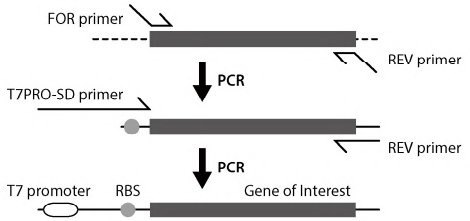
For the circular DNA, e.g. plasmid DNA, T7 terminator is required at the downstream of stop codon.

For the linear DNA, e.g. PCR product or DNA fragment digested by restriction enzymes, T7 terminator is not necessarily required.

Template DNA

2. Prepatation of the template DNA

An example of the method for preparing the template DNA by two-step PCR is shown in below.



3. Sequence of primers

FOR primer

5′–AAGGAGATATACCA–ATG–N(10–20)–3′
RBS

REV primer

5′–GGATTAGTTATTCA–TTA–N(10–20)–3′
more than 10 any nucleotides

T7PRO-SD primer

5′–GAAATTAATACGACTCACTATAGGGAGACCACAACGGTTTCCC
T7 promoter
TCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACCA–3′
RBS

Kit components

- **Solution I** *1 — 5x 100 µL
  Amino acids, NTPs, tRNAs and substrates of enzymes
  Store at -20ºC
- **Solution II** *2 — 5x 12.5 µL
  Proteins in 30% glycerol buffer
  Store at -20ºC or -80ºC *3
- **Solution III** *2 — 5x 25 µL
  Ribosomes (20 µM)
  Store at -80ºC *3
- **Cysteine** *1 — 5x 20 µL
  Cysteine (10 mM)
  Store at -20ºC
- **DTT** *1 — 5x 20 µL
  Dithiothreitol (40 mM)
  Store at -20ºC
- **GSH** *1 — 5x 20 µL
  Reduced glutathione (80 mM)
  Store at -20ºC
- **DHFR DNA** *2, 4 — 5x10 µL
  PCR product (20 ng/µL) containing a gene encoding *E. coli* DHFR
  Store at -20ºC

Kit components

Store at -80 ºC before opening

*1)
Reducing agents are excluded from Solution I in PURE*frex*® *2.1*. Please add attached Cysteine and reducing agent (DTT or GSH) to the reaction mixture.
The standard working concentration of Cysteine, DTT and GSH are 0.5, 2 and 4 mM, respectively. Other reducing agents are also available for the reaction, e.g. 2-mercaptoethanol and TCEP.
We recommend optimizing the concentration of reducing agent to synthesize a protein with higher activity because the suitable concentration is depends on the synthesized protein.

*2)
Solution II, Solution III and DHFR DNA are the same as PURE*frex*® *2.0* (#PF201).

*3)
For storage at -80ºC, the remaining 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.

*4)
As a positive control for the protein synthesis reaction, 1 µL of DHFR DNA should be added to 20 µL of reaction. Please visit our web site to get the nucleic acid sequence of DHFR DNA.

Protocol

The protein synthesis reaction using PURE*frex*® *2.1* can be performed in any volume. For example, 20 µL of reaction mixture containing 0.5 mM Cysteine and 4 mM GSH is assembled as below.

1. Thaw completely Solution I, Cysteine and GSH by incubation at room temperature or 37 ºC for 1 minute, and then cool on ice.
2. Thaw Solution II and Solution III 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	7 -X µL
Solution I	8 µL*5)
Cysteine (10 mM)	1 µL
GSH (80 mM)	1 µL
Solution II	1 µL
Solution III	2 µL
Template DNA	X µL
Total	20 µL

5. Incubate the tube at 37ºC for 2-6 hours.
6. Analyze the synthesized product.

*5)
Please note that the volume of Solution I in PURE*frex*® *2.1* (#PF213) is different from PURE*frex*® *2.0* (#PF201).

Note

PURE*frex*® is developed for in vitro research use only. PURE*frex*® should not be used for 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 water, reagents and equipment should be used. We also recommend wearing gloves and mask.

For information concerning commercial use of PURE*frex*®, please contact GeneFrontier.
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