Reconstituted cell-free protein synthesis kit

PURE*frex*® 2.0

#PF201-0.25-5-EX

For 5x 250 µL Reaction

in vitro research use only

Store at -80°C before opening

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Introduction

1. Overview

PURE*frex*® is a reconstituted cell-free protein synthesis kit which GeneFrontier has developed based on the PURE system technology. The PURE system is a cell-free protein synthesis system, which has originally developed by Professor Takuya Ueda at the University of Tokyo, and is consisted of only purified factors necessary for transcription, translation and energy regeneration (Ref. 1). The target protein is synthesized by adding the template DNA (or mRNA) to the reaction mixture. PURE*frex*® is consisted of only purified factors, therefore it enables to adjust the composition of the reaction mixture.

PURE*frex*® is raised in the purity by improving the preparation methods of 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 below 0.1 EU per 1 µL of reaction and other contaminants, such as RNase and β-galactosidase, are also reduced.

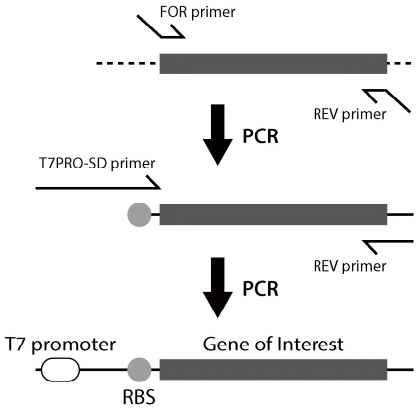
In the PURE*frex*®, all proteins have no tags for purification or detection, therefore the target protein would be synthesized and purified by any tag.

References) 1. Shimizu *et al.* (2001) *Nat. Biotecnol.*, vol. 19, p. 751
2. Shimizu *et al.* (2005) *Methods*, vol. 36, p. 299

Template DNA

2-2. Preparation of the template DNA

An example of preparation of the template DNA by PCR is shown below. Nucleic acid sequences of the primers are shown in 2-3. The elements shown in 2-1 are necessary for the template DNA, even using the other preparing methods.



Protocol

The protein synthesis reaction using PURE*frex*® is scalable. For example, 20 µL of reaction is assembled as below.

1. Thaw Solution I by incubation at 30°C for 1 minute, and then cool on ice.
2. Thaw Solution II and III on ice.
3. Mix Solution I, II and III 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	7 -X µL
Solution I	10 µL
Solution II	1 µL
Solution III	2 µL
Template DNA	X µL
Total	20 µL

5. Incubate the tube at 37°C for 2-4 hours.
6. Analyze the synthesized product.

Kit components

Store at -80°C before opening

Solution I (White) **5x 125 µL**

Amino acids, NTPs, tRNAs and substrates for enzymes

Store at -20°C

Solution II (Black) **5x 12.5 µL**

Proteins in 30% glycerol buffer

Store at -20°C or -80°C*1

Solution III (Red) **5x 25 µL**

Ribosomes (20 µM)

Store at -80°C*1

DHFR DNA (Clear)*2 **5x 10 µL**

PCR product (20 ng/µL) containing a gene encoding *E. coli* DHFR

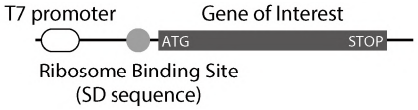
Store at -20°C

*1)
For storage at -80°C, the rest of solution should be frozen rapidly in liquid nitrogen or dry ice/ethanol. Divide into aliquots, if necessary, and avoid refreeze and thaw as much as possible.

*2)
As a positive control for the protein synthesis reaction, 1.0 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.

Template DNA

2-1. Construct 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. All three stop codons are available. More than 10 nucleotides are needed after the stop codon.

Both circular and linear DNAs are available as a template DNA. For the circular DNA, T7 terminator is required at the downstream of the stop codon. For the linear DNA, which includes a PCR product and a DNA digested by a restriction enzyme, T7 terminator is not necessarily required at the 3'-terminus.

Template DNA

2-3. Sequences 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′-GAAATTAATACGACTCACTATAGGGAGACC
T7 promoter
ACAACGGTTTCCCTCTAGAAATAATTTTGTTTA
ACTTTAAGAAGGAGATATACCA-3′
RBS

Note

PURE*frex*® is developed for *in vitro* research use only. 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 PURE*frex*®, please contact GeneFrontier (purefrex@genefrontier.com).

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