Reconstituted cell-free protein synthesis kit

PURE*frex*®

#PF001

For 500 µL Reaction

Lot :

Expiry Date :

in vitro research use only

August 2011

GeneFrontier Corporation
www.genefrontier.com
Todai-Kashiwa VP308
5-4-19 Kashiwanoha, Kashiwa-shi,
Chiba 277-0882 Japan

Description

1. Overview

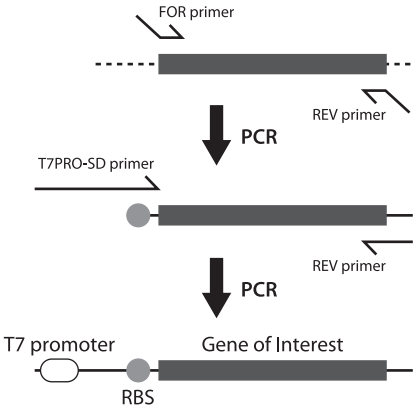
PURE*frex*® kit is a newly developed reconstituted cell-free protein synthesis reagent based on the PURE system technology invented by Professor Takuya Ueda in the University of Tokyo. This reaction system consists of proteins, ribosome, tRNA, amino acids and NTPs necessary for transcription, translation and energy regeneration (Ref. 1, 2). The proteins and ribosome are highly purified individually and assembled together to constitute the protein synthesis system. To synthesize your protein, just add your template DNA or mRNA encoding the protein of interest into the reaction mixture, and incubate for several hours. This system's biggest point is a RECONSTITUTED system by assembling translation-related factors only. By this unique character, you may adjust the composition of the reaction mixture as you like and may not have to consider serious background for your downstream application.

By improving the purification process of components of PURE*frex*® kit, contamination of RNase and β-galactosidase are greatly reduced, in addition to that, lipopolysaccharide is also reduced less than 0.1 EU per 1 µL of the reaction mixture. All proteinous components in PURE*frex*® kit have no tags for purification and detection. It allows to fuse your protein with any tag to purify the product.

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

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, 50 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 1-3 ng/µL per 1 kb)

Water	20-X µL
Solution I	25 µL
Solution II	2.5 µL
Solution III	2.5 µL
Template DNA	X µL
Total	50 µL

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

Kit components

Solution I (Blue) **250 µL**

Amino acids, NTPs, tRNAs and substrates for enzymes

Store at -20°C

Solution II (Yellow) **25 µL**

Proteins in 30% glycerol buffer

Store at -20°C or -80°C *1

Solution III (Red) **25 µL**

Ribosomes (20 µM)

Store at -80°C *1

DHFR DNA *2 **10 µL**

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

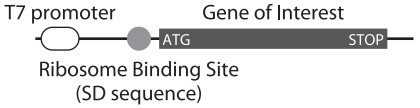
Store at -20°C

*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, 2.5 µL of DHFR DNA should be added to 50 µL of reaction. Please visit our web site to get the nucleic acid sequence of DHFR DNA.

2. 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.

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.

Support

For inquiry about the PURE*frex*®, please contact our Technical Service.

PURE*frex*® Technical Service
E-mail: purefrex@genefrontier.com