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Reconstituted cell-free protein synthesis kit

PURE*frex*® 1.0

#PF001-0.25-EX For 250 µL Reaction

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

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 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	0.25		
• **Solution I**	**125 µL**	×1		
	Amino acids, NTPs, tRNAs and substrates of enzymes Store at -20ºC			
• **Solution II**	**12.5 µL**	×1		
	Proteins in 30% glycerol buffer Store at -20ºC or -80ºC *1			
• **Solution III** *2	**12.5 µL**	×1		
	Ribosomes (20 µM) Store at -80ºC *1			
• **DHFR DNA** *2, 3	**10 µL**	×1		
	PCR product (20 ng/µL) containing a gene encoding *E. coli* DHFR 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.
*2) Solution III and DHFR DNA are the same as PURE*frex*® *2.0* (#PF201) and PURE*frex*® *2.1* (#PF213).
*3) 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.

How to aliquot

PURE*frex*® kits are highly stable during storage at -80°C. However, repeated freeze-thaw cycles can lead to a reduction in activity. If only small amounts are required per use, we recommend aliquoting the product into smaller portions for storage. When aliquoting small volumes, using low-binding tubes is advisable to minimize loss and ensure reliability.

Solution I

Thaw the solution by incubating it at room temperature or 37°C for approximately 5 minutes and mix thoroughly until it becomes completely clear. Aliquot the solution without placing it on ice and freeze the aliquots at -20°C or below.

Solution II

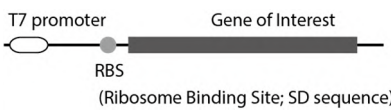
Thaw the solution on ice and mix thoroughly (for small volumes, gentle vortexing is acceptable as long as it does not create bubbles). Aliquot the solution and rapidly freeze the aliquots using liquid nitrogen or a freezing mixture of dry ice and ethanol. Finally, store them at -20°C or below.

Solution III

Thaw the solution on ice, avoiding extended exposure to the thawed state. Mix thoroughly, aliquot the solution, and rapidly freeze using liquid nitrogen or a freezing mixture of dry ice and ethanol. Store the aliquots at -80°C. Avoid freezing directly in a -80°C freezer without rapid freezing, as this can result in decreased activity.

Template DNA

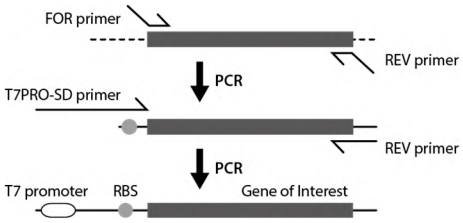
1. Construction of the template DNA



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.

2. Preparation 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

Protocol

The protein synthesis reaction using PURE*frex*® *1.0* can be performed in any volume. For example, 20 µL of reaction is assembled as below.

1. Thaw Solution I by incubation at room temperature or 37 °C for 5 minutes for completely dissolving, and then leave at room temperature.
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	8-X	µL
Solution I	10	µL
Solution II	1	µL
Solution III	1	µL
Template DNA	X	µL
Total	20	µL

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

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.
PURE*frex*® is a registered trademark of GeneFrontier Corporation. For any commercial use of PURE*frex*®, please contact GeneFrontier in advance.

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