

**PUREfreflex® 2.0 mini**

#PF201-0.1-EX

For 100 µL Reaction

*in vitro* research use only

Store at -80°C before opening

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GeneFrontier Corporation  
www.genefrontier.comSHARP Kashiwa Building, 4F  
273-1 Kashiwa, Kashiwa-shi,  
Chiba 277-0005 Japan**Introduction****About PUREfreflex®**

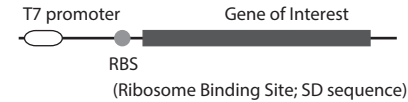
PUREfreflex® is a reconstituted cell-free protein synthesis kit which GeneFrontier has developed based on the PURE system technology. The target protein can be synthesized by adding the template DNA (or mRNA) to the reaction mixture. The PURE system is a unique cell-free protein synthesis system, which 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.

PUREfreflex® 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 PUREfreflex® 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 PUREfreflex® 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.

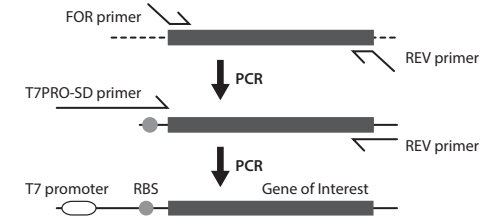
Both circular and linear DNAs are available as a template DNA.

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. 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  
TCTAGAAATAAATTTTGTAACTTTAAGAAGGAGATATACCA-3'  
RBS

**Kit components**

- Solution I** 50 µL  
Amino acids, NTPs, tRNAs and substrates of enzymes  
Store at -20°C
- Solution II** \*1 5 µL  
Proteins in 30% glycerol buffer  
Store at -20°C or -80°C \*3
- Solution III** \*1 10 µL  
Ribosomes (20 µM)  
Store at -80°C
- DHFR DNA** \*2 10 µL  
PCR product (20 ng/µL) containing a gene encoding *E. coli* DHFR  
Store at -20°C
- T7PRO-SD primer** 5 µL  
PCR primer (10 µM) for preparation of the template DNA  
Store at -20°C
- RNase-Free Water** 500 µL  
Store at -20°C

**Kit components**

- \*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.

**Protocol**

PUREfreflex® 2.0 mini Instruction Manual

<https://purefreflex.genefrontier.com/media-download/548/33f54c90148b4952/>**Protocol**

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

1. Thaw Solution I by incubation at room temperature or 37 °C for 1 minute completely, 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 2-6 hours.
6. Analyze the synthesized product.

**Note**

PUREfreflex® is developed for *in vitro* research use only. PUREfreflex® 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.

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