Introduction

1. Overview
PUREflex® is a reconstituted cell-free protein synthesis kit which GeneFrontier has developed based on the PUREflex 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 consists of only purified factors necessary for transcription, translation and energy regeneration (Ref. 3). The target protein is synthesized by adding the template DNA (or mRNA) to the reaction mixture. PUREflex® is consists of only purified factors, therefore it enables to adjust the composition of the reaction mixture.

PUREflex® 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 contaminates, such as RNase and β-galactosidase, are also reduced.

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

References

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 2-4. The elements shown in 2-1 are necessary for the template DNA, even using the other preparing methods.

Template DNA

2-3. Sequences of primers
FOR primer
5’-AAGGAGATAATCCA-ATG-N (10 -20) -3’

REV primer
5’-GTTAGTACCTTCTA-N (10 -20) -3’

ACACTTGGTATCCCTTCTAGATATTGTTA
ACCTTAAAGGAGATAATACCA-3’

FOR primer

Note

PUREflex® is developed for in vitro research use only. PUREflex® 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 gowns and mask.

For information concerning commercial use of PUREflex®, please contact GeneFrontier (pureflex@genefrontier.com).

Distributor

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Kit components

Solution I (White) 125 µL
Amino acids, NTPs, tRNAs and substrates for enzymes
Store at -20°C

Solution II (Black) 12.5 µL
Proteins in 30% glycerol buffer
Store at -20°C or -80°C*

Solution III (Red) 25 µL
Ribosomes (20 µM)
Store at -80°C*

DHFR DNA (Clear) 10 µL
PCR product (20 ng/µL) containing a gene encoding E. coli DHFR
Store at -20°C

* For storage at -40°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 At 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.