GroE Mix

#PF004-0.5-EX

For 500 µL Reaction

PURE*frex*® is NOT included.

in vitro research use only

Store at -80ºC before opening

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Introduction

1. Overview

GroE Mix is a newly developed supplement of PURE*frex*® to assist proper folding and solubility of your protein.

PURE*frex*® is a cell-free protein synthesis reagent but has no molecular chaperones (See next page).

When your protein of interest needs molecular chaperones for proper protein folding, GroE Mix could be a solution for that.

GroE Mix is constituted of highly purified GroEL (known as Hsp60) and GroES (working in conjunction with GroEL) from *E.coli* with the optimized ratio.

GroE Mix works very well with PURE*frex*® (#PF001-0.25 or #PF201-0.25) in a single tube for protein synthesis reaction, which could lead to the preparation of your protein in proper folding with good solubility.

Introduction

2. About PURE*frex*®

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

Note

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

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Kit components

Store at -80ºC before opening

- **GroE Mix (Purple)** **12.5 µL**
  20µM GroEL and 40µM GroES in 30% glycerol buffer[*1]
  Store at -80ºC[*2]

- **Dilution Buffer (Clear)** **500 µL**
  30% glycerol buffer
  Store at -20ºC

*1)
GroEL 14-mer and GroES 7-mer forms

*2)
For storage at -80ºC, 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.

Protocol

GroE Mix is worked with PURE*frex*® (#PF001-0.25、#PF201-0.25) in one tube. For example, 20 µL of reaction is assembled as below.

1. Thaw Solution I by incubation at 30ºC for 1 minute, and then cool on ice.
2. Thaw Solution II, III and GroE Mix on ice.
3. Mix Solution I, II, III and GroE Mix by vortex and centrifuge briefly to collect each solution at the bottom.
4. Assemble the reaction mixture in a tube as follows. Do not add GroE Mix at this step.

   (Add the template DNA to 0.5-3 ng/µL per 1 kbp)

	#PF001	#PF201
Water	8-X µL	7-X µL
Solution I	10 µL	10 µL
Solution II	1 µL	1 µL
Solution III	1 µL	2 µL
Template DNA	X µL	X µL
Total	19 µL	19 µL

Protocol

5. Incubate the tube at 37ºC for 15 minutes.[*3]
6. Make a Two-fold dilution of GroE Mix with Dilution Buffer and add 1 µL to incubated tube in Step5.[*4]
7. Incubate the tube at 37ºC for 2-4 hours.
8. Analyze the synthesized product.

*3)
To prevent an inhibition of transcription, we recommend a pre-incubation before addition of GroE Mix.

*4)
The optimum concentration of GroE Mix depends on protein of interest. Please use Dilution buffer for dilution of GroE Mix.

Memo