Mar 2025

GroE Mix

#PF004-10ML-EX For 2 mL x 5 Reaction
#PF004-50ML-EX For 2 mL x 25 Reaction

(PURE*frex*® is NOT included.)

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

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 section). 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, #PF201 or #PF213) in a single tube for protein synthesis reaction, which could lead to the preparation of your protein in proper folding with good solubility.

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 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	10ML	50ML	
• **GroE Mix (Purple)**	**50 µL**	×5	×25	
	20 µM GroEL and 40 µM GroES in 30% glycerol buffer *1 Store at -80ºC *2			
• **Dilution Buffer (Clear)**	**500 µL**	×1	×1	
	30% glycerol buffer Store at -20ºC			

Store at -80ºC before opening

*1) GroEL 14-mer and GroES 7-mer forms.
*2) 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.

Protocol

GroE Mix works very well with PURE*frex*® (#PF001, #PF201 or #PF213) in a single tube. 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, III and GroE Mix 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)

	#PF001		#PF201	
Water	7-X	µL	6-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

	#PF213	
Water	6-X	µL
Solution I	8	µL *3
Cysteine	1	µL
GSH	1	µL
Solution II	1	µL
Solution III	2	µL
Template DNA	X	µL
Total	19	µL

5. Incubate the tube at 37ºC for 15 minutes. *4
6. Make a Two-fold dilution of GroE Mix with Dilution Buffer and add 1 µL to incubated tube in Step5. *5
7. Incubate the tube at 37ºC for 2-4 hours (#PF001) or 4-6 hours (#PF201 or #PF213).
8. Analyze the synthesized product.

*3) Please note that the volume of Solution I in PURE*frex*® *2.1* (#PF213) is different from PURE*frex*® *2.0* (#PF201).
*4) To prevent inhibition of transcription, we recommend a pre-incubation at 37ºC for 15 minutes before addition of GroE Mix.
*5) The standard concentration of GroE Mix is 0.5 µM GroEL and 1 µM GroES. The optimal concentration of GroE Mix depends on the protein of interest. Please use Dilution Buffer for dilution of GroE Mix.

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 any commercial use of GroE Mix, please contact GeneFrontier in advance.
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