

# DnaK Mix

**#PF003-10ML-EX** For 2 mL x 5 Reaction  
**#PF003-50ML-EX** For 2 mL x 25 Reaction

(PUREfreflex® is NOT included.)

*In vitro* research use only  
 Store at -80°C before opening

## Introduction

### 1. Overview

DnaK Mix is a newly developed supplement of PUREfreflex® to assist proper folding and solubility of your protein. PUREfreflex® 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, DnaK Mix could be a solution for that.

DnaK Mix is constituted of highly purified DnaK, DnaJ and GrpE from *E. coli* with the optimized ratio. DnaK known as Hsp70 has ATPase activity and is stimulated by co-chaperones, DnaJ and GrpE. DnaJ facilitates the ATPase activity of DnaK and could bind to a hydrophobic region of protein. GrpE stimulates ADP/ATP exchange rate of DnaK.

DnaK Mix works very well with PUREfreflex® (#PF001, #PF201 or #PF213) and with DsbC Set (#PF005) or PDI Set (#PF006) 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 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 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 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

## Kit components

	Volume	10ML	50ML	
• DnaK Mix (Orange)	100 µL	×5	×25	
	100 µM DnaK, 20 µM DnaJ, 20 µM GrpE in 30% glycerol buffer Store at -80°C *1			
• Dilution Buffer (Clear)	500 µL	×1	×1	
	30% glycerol buffer Store at -20°C			

Store at -80°C before opening

\*1) For storage at -80°C, 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

DnaK Mix works very well with PUREfreflex® (#PF001, #PF201 or #PF213) and with DsbC Set (#PF005) or PDI Set (#PF006) 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 DnaK 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
DnaK Mix *2	1 µL	1 µL
Template DNA	X µL	X µL
Total	20 µL	20 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
DnaK Mix *2	1 µL
Template DNA	X µL
Total	20 µL

5. Incubate the tube at 37°C for 2-4 hours (#PF001) or 4-6 hours (#PF201 or #PF213).
6. Analyze the synthesized product.

\*2) The standard concentration of DnaK Mix is 5 µM DnaK, 1 µM DnaJ and 1 µM GrpE. The optimal concentration of DnaK Mix depends on the protein of interest. Please use Dilution Buffer for dilution of DnaK Mix.

\*3) Please note that the volume of Solution I in PUREfreflex® 2.1 (#PF213) is different from PUREfreflex® 2.0 (#PF201).

## Note

DnaK Mix is developed for *in vitro* research use only. DnaK 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 DnaK Mix, please contact GeneFrontier in advance.  
 e-mail : purefreflex@genefrontier.com

## Distributor

 COSMO BIO USA

[Outside Japan]  
 2792 Loker Ave West, Suite 101, Carlsbad, CA 92010, USA  
 email: info@cosmobiousa.com  
 Phone/FAX: (+1) 760-431-4600  
 URL: www.cosmobiousa.com

 COSMO BIO CO., LTD.

[JAPAN]  
 TOYO EKIMAE BLDG. 2-20, TOYO 2-CHOME,  
 KOTO-KU, TOKYO 135-0016, JAPAN  
 Phone: +81-3-5632-9610 FAX: +81-3-5632-9619  
 URL: https://www.cosmobio.co.jp/

## Manufacturer

 GeneFrontier  
 www.genefrontier.com

GeneFrontier Corporation  
 SHARP Kashiwa Building, 4F, 273-1 Kashiwa,  
 Kashiwa-shi, Chiba 277-0005 Japan