DnaK Mix

#PF003-0.5-EX

For 500 µL Reaction

Lot :

Expiry Date :

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

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Introduction

1. Overview

DnaK 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, 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 PURE*frex*® (#PF001-0.25 or #PF201-0.25) (and with DS supplement (#PF005-0.5)) 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

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 information concerning commercial use of DnaK Mix, please contact GeneFrontier (purefrex@genefrontier.com).

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Kit components

Store at -80ºC before opening

DnaK Mix (Orange) 25 µL

100µM DnaK, 20µM DnaJ and 20µM GrpE in 30% glycerol buffer

Store at -80ºC*1

Dilution Buffer (Clear) 500 µL

30% glycerol buffer

Store at -20ºC

*1)
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

DnaK Mix is worked with PURE*frex*® (#PF001-0.25、#PF201-0.25) or DS supplement (#PF005-0.5) 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 DnaK Mix on ice.
3. Mix Solution I, II, III and DnaK Mix 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)

	#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

Protocol

5. Incubate the tube at 37ºC for 2-4 hours.
6. Analyze the synthesized product.

*2)
The optimum concentration of DnaK Mix depends on protein of interest. Please use Dilution buffer for dilution of DnaK Mix.

Memo