Performance and application of PURE*frex*® 2.0 with increased productivty

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Abstract

The PURE (Protein synthesis Using Recombinant Elements) system is a reconstituted cell-free protein synthesis system, which consists of only purified factors necessary for transcription, translation and energy regeneration. The PURE system has the unique features that it contains less contaminant such as nucleases and proteases and that composition of the reagents can be easily adjusted in accordance to the purpose. We modified the preparation methods of all components that were purified from *Escherichia coli* and developed the new PURE system, which was launched as "PURE*frex*®" in 2011. In PURE*frex*, the produced proteins can be directly applied to cell-based assay without any purification because the quantity of contaminating LPS is remarkably reduced.

Here, we report that we improved the protein productivity of PURE*frex* and named the advanced version of PURE*frex* as PURE*frex* 2.0. PURE*frex* 2.0 achieved 2-10 times higher protein productivity than the current version of PURE*frex* (PURE*frex* 1.0). The productivity of *E. coli* dihydrofolate reductase and green fluorescent protein reached to approximately 1 mg/mL in batch mode using PURE*frex* 2.0. We also succeeded to synthesize active proteins, which require formation of disulfide bonds or molecular chaperones for correct folding, using PURE*frex* 2.0 with oxidized glutathione (GSSG) and/or appropriate molecular chaperones.

PURE*frex* 2.0 will be a useful tool for *in vitro* production of functional proteins such as an antibody, a protein based toxin or an immunotoxin, and vaccines etc.

Summary

E. coli based cell-free protein sysnthesis systems

	Extract system	Reconstituted system		
	S30 system	PURE system (original)	PURE*frex*®	PURE*frex*®2.0
Typical Yield (μg/mL)	100-1,000	10-200	10-200	20-1,000
Contamination				
RNase	very High	Low	very Low	very Low
LPS	very High	High	very Low	very Low
Template DNA				
Plasmid DNA	OK	OK	OK	OK
PCR product	NG	OK	OK	OK
Customization of Reagent	Difficult	Easy	Easy	Easy
Purification of His-tagged product	OK	NG	OK	OK

PURE*frex*® • PURE*frex*®2.0

a regular kit for the synthesis of proteins without disulfide bonds

PURE*frex*®*DS* • PURE*frex*®*DS*2.0

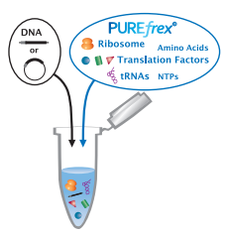
a kit for the synthesis of proteins containing disulfide bonds

DnaK Mix • GroE Mix

a supplement for the synthesis of aggregate-prone proteins

1. PURE*frex*®; based on the PURE system technology

The PURE system is a reconstituted cell-free protein synthesis system, which consists of only purified factors necessary for transcription, translation and energy regeneration.



Advantage

- Low level of contamination
- Easy adjustment of the reagent composition
- PCR products usable as a temlplate DNA

Application

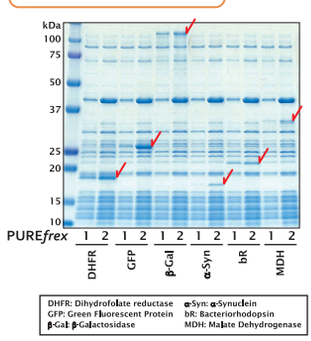
- High throughput preparation of proteins (including Fab, scFv, protein toxin etc.)
- Protein science research
- *in vitro* display
  - Ribosome display
  - mRNA display

(Ref; Shimizu Y. *et al.* (2001) *Nat. Biotechnol.*, vol. 19, p. 751)

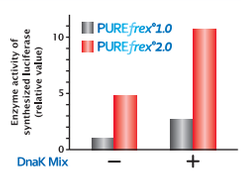
2. Performance of PURE*frex* 2.0

PURE*frex* 2.0 has 2-10 times higher productivity than PURE*frex* 1.0.

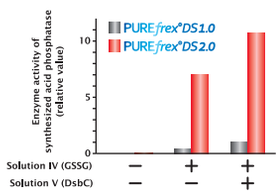
PURE*frex*®1.0 or PURE*frex*®2.0
↓ + template DNA (PCR product)
↓ incubation at 37°C for 4 h
↓ SDS-PAGE (1 μL of reaction/lane)
↓ CBB staining



Aggregate-prone proteins can be synthesized maintaining their activity.



Proteins containing disulfide bonds can be synthesized in active form.

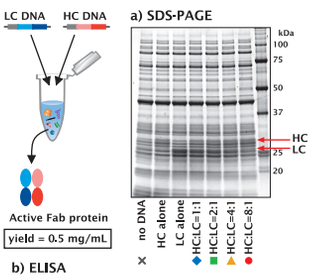


3. Application of PURE*frex* 2.0; can be applicable for synthesis/screening for Fab, Protein toxin and Immunotoxin

Fab

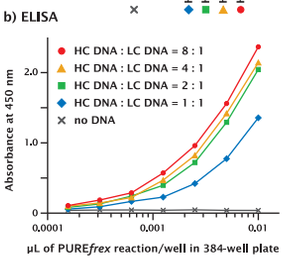
Fab protein can be synthesized in active form from separate template DNA.

PURE*frex*®*DS*2.0
↓ + HC and LC DNA (PCR product) from Herceptin with each ratio
↓ incubation at 37°C for 4 h
directly applied to
↓ a) SDS-PAGE (0.5 μL of reaction/lane) and Oriole staining
b) ELISA



a) SDS-PAGE

b) ELISA

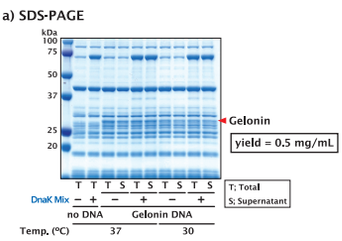


Protein Toxin

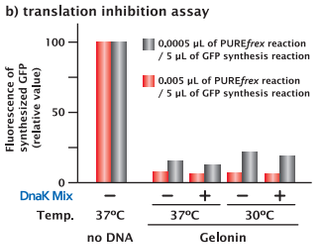
Protein toxin can be synthesized, showing the activity *in vitro*.

PURE*frex*®2.0 +/- DnaK Mix
↓ + Protein Toxin (Gelonin*) DNA (PCR product)
↓ incubation at 30 or 37°C for 4 h
↓ spin to separate the supernatant
directly applied to
↓ a) SDS-PAGE (1 μL of reaction/lane) and CBB staining
b) translation inhibition assay

a) SDS-PAGE



b) translation inhibition assay

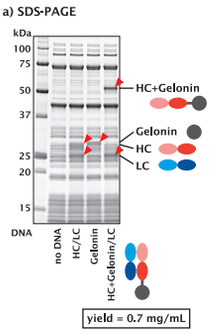


Immunotoxin (Fab + Toxin)

Immunotoxin (Fab+Toxin) can be synthesized, showing the cytotoxicity *in vitro*.

PURE*frex*®*DS*2.0 + DnaK Mix
↓ + HC & LC, Gelonin, HC+Gelonin & LC DNA (PCR product)
↓ incubation at 37°C for 4 h and spin
directly applied to
↓ a) SDS-PAGE (0.5 μL of reaction/lane) and Oriole staining
b) ELISA
c) translation inhibition assay
d) cell growth inhibition assay

a) SDS-PAGE



translation inhibition assay

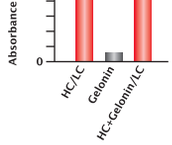
HeLa cell-extract based cell-free protein synthesis system
↓ + synthesized products with PURE*frex*
↓ + GFP DNA
↓ incubation at 37°C for 1.5 h
↓ measurement of fluorescence of GFP

cell growth inhibition assay

SK-BR-3 cell (Her2 positive cell)
↓ + synthesized products with PURE*frex* in culture medium
↓ incubation for 3 days
↓ cell proliferation assay by WST reagent

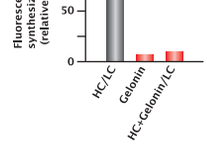
b) ELISA

0.00125 μL of PURE*frex* reaction /well in 384-well plate



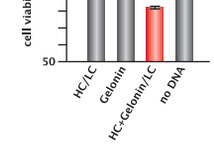
c) translation inhibition assay

0.005 μL of PURE*frex* reaction /5 μL of GFP synthesis reaction



d) cell growth inhibition assay

0.0625 μL of PURE*frex* reaction /well in 96-well plate



*Gelonin
; is a 30-kDa protein from plant *Gelonium multiflorum*.
; is one of ribosome-inactivating protein (RIP).
; has rRNA N-glycosidase activity to eukaryote ribosome.

On-going

- Developing the system for manufacturing purpose.
- Developing a novel protein toxin with very high cytotoxicity.

PURE*frex*® / PURE*frex*®2.0

For more information, please contact us.
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