

Performance and application of PUREfrex® 2.0 with increased productivity

Takashi Kanamori, Satoshi Murakami, Rena Matsumoto, Mikiko Nakamura
GeneFrontier Corporation (Chiba, Japan)

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 "PUREfrex®" in 2011. In PUREfrex, 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 PUREfrex and named the advanced version of PUREfrex as PUREfrex 2.0. PUREfrex 2.0 achieved 2-10 times higher protein productivity than the current version of PUREfrex (PUREfrex 1.0). The productivity of *E. coli* dihydrofolate reductase and green fluorescent protein reached to approximately 1 mg/mL in batch mode using PUREfrex 2.0. We also succeeded to synthesize active proteins, which require formation of disulfide bonds or molecular chaperones for correct folding, using PUREfrex 2.0 with oxidized glutathione (GSSG) and/or appropriate molecular chaperones.

PUREfrex 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 synthesis systems

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

PUREfrex® • PUREfrex® 2.0

a regular kit for the synthesis of proteins without disulfide bonds

PUREfrex® DS • PUREfrex® DS 2.0

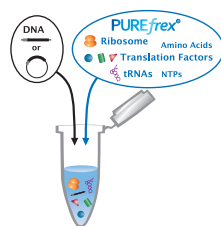
a kit for the synthesis of proteins containing disulfide bonds

DnaK Mix • GroE Mix

a supplement for the synthesis of aggregate-prone proteins

1. PUREfrex®; 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 template DNA

Application

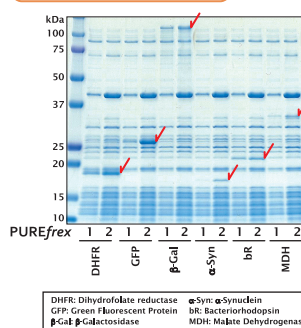
- High throughput preparation of proteins (including Fab, scFv, protein toxin etc.)
- Protein science research
- *in vitro* display
- Ribosome display
- mRNA display

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

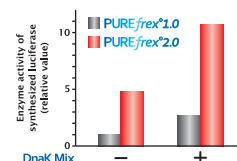
2. Performance of PUREfrex 2.0

PUREfrex 2.0 has 2-10 times higher productivity than PUREfrex 1.0.

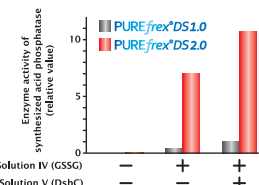
PUREfrex® 1.0 or PUREfrex® 2.0
↓ + template DNA (PCR product)
↓ incubation at 37°C for 4 h
↓ SDS-PAGE (1 μL of reaction/lane)
↓ CBB staining



Aggregate-prone proteins can be synthesized maintaining their activity.



Proteins containing disulfide bonds can be synthesized in active form.

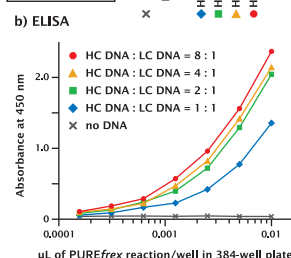
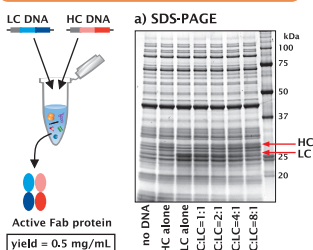


3. Application of PUREfrex 2.0; can be applicable for synthesis/screening for Fab, Protein toxin and Immunotoxin

Fab

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

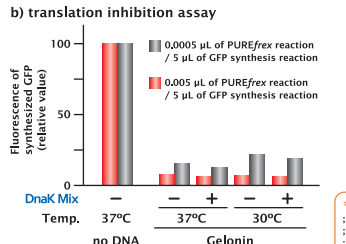
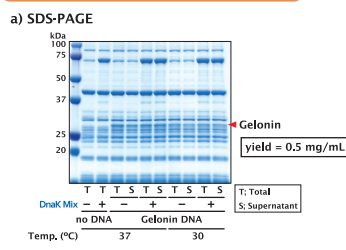
PUREfrex® DS 2.0
↓ + HC and LC DNA (PCR product) from Herceptin with each ratio
↓ incubation at 37°C for 4 h
↓ directly applied to
a) SDS-PAGE (0.5 μL of reaction/lane) and Oriole staining
b) ELISA



Protein Toxin

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

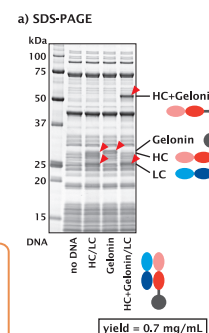
PUREfrex® 2.0 +/- DnaK Mix
↓ + Protein Toxin (Gelolin™) DNA (PCR product)
↓ incubation at 30 or 37°C for 4 h
↓ spin to separate the supernatant
↓ directly applied to
a) SDS-PAGE (1 μL of reaction/lane) and CBB staining
b) translation inhibition assay



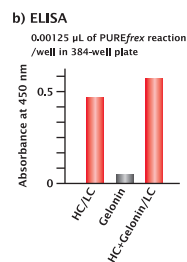
Immunotoxin (Fab + Toxin)

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

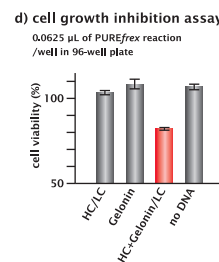
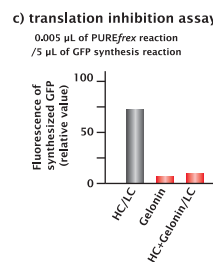
PUREfrex® DS 2.0 + DnaK Mix
↓ + HC & LC, Gelolin, HC+Gelolin & LC DNA (PCR product)
↓ incubation at 37°C for 4 h and spin
↓ directly applied to
a) SDS-PAGE (0.5 μL of reaction/lane) and Oriole staining
b) ELISA
c) translation inhibition assay
d) cell growth inhibition assay



translation inhibition assay
HeLa cell-extract based cell-free protein synthesis system
↓ + synthesized products with PUREfrex
↓ + 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 PUREfrex in culture medium
↓ incubation for 3 days
↓ cell proliferation assay by WST reagent



^a Gelolin
: 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.