

Performance of PUREfrex® 2.0 with increased productivity and its application

生産量を増加させた再構成型タンパク質合成系(PUREfrex® 2.0)の性能と利用

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Abstract

PURE systemは、タンパク質合成に必要な因子を個別に精製して再構成した無細胞タンパク質合成系であり、合成を阻害するRNaseやプロテアーゼなどの混入量が少なく、使用目的に合わせて反応液組成を自由に調整できる、などの優れた特長を有している。我々は、PURE systemを基に、His-tagなしの(タンパク質)因子で再構成され、大腸菌由来のリボ多糖混入量を1/1,000以下に減少させたPUREfrex®を完成、製品化した。PUREfrex®は、PURE systemの特長を強化した反応系で、Ribosome Display等の利用に好適であるが、細胞抽出液を利用する無細胞タンパク質合成系に比べるとタンパク質合成量が低いことが課題であった。そこで我々は、PUREfrex®のタンパク質合成能を増強させることを目的に反応液組成の最適化検討を再度行った。その結果、従来のPUREfrex(PUREfrex® 1.0)比で、単位体積当たり10倍量のGFPを合成できるPUREfrex® 2.0を完成させることができた。さらにPUREfrex® 2.0は、PUREfrex® 1.0と同様、ジスルフィド結合を含むタンパク質や凝集しやすいタンパク質も、系中に適切な因子を添加して合成することで高い活性を有したタンパク質として合成可能であった。また、本発表では、PUREfrex® 2.0を抗体断片のFabやscFvの合成に適用した事例についても報告する。

PUREfrex® 1.0 • PUREfrex® 2.0

a regular kit for the synthesis of proteins without disulfide bonds

DS supplement

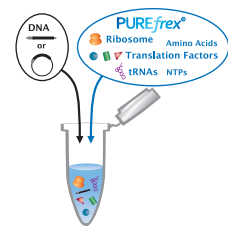
a supplement for the synthesis of proteins containing disulfide bonds

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)

Summary

E. coli based cell-free protein synthesis systems

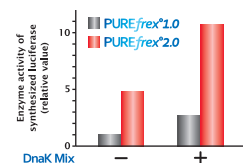
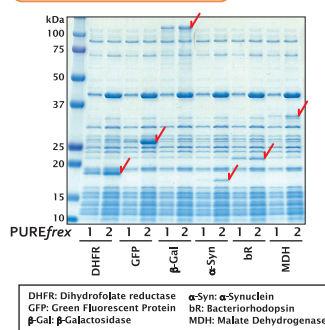
	Extract system	Reconstituted system		
	S30 system	PURE system (original)	PUREfrex® 1.0	PUREfrex® 2.0
Typical Yield (µg/mL)	100-1,000	10-200	10-200	20-1,000
Contamination RNase LPS	very High very High	Low High	very Low very Low	very Low very Low
Template DNA Plasmid DNA PCR product	OK NG	OK OK	OK OK	OK OK
Customization of Reagent	Difficult	Easy	Easy	Easy
Purification of His-tagged product	OK	NG	OK	OK

2. Performance of PUREfrex 2.0

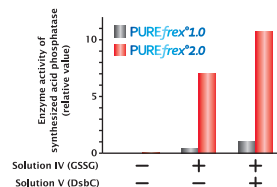
PUREfrex 2.0 has 2-10 times higher productivity than PUREfrex 1.0.

Aggregate-prone proteins can be synthesized maintaining their activity.

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



Proteins containing disulfide bonds can be synthesized in active form.



3. Application of PUREfrex 2.0; can be applicable for synthesis/screening for Fab and scFv.

The nucleotide sequence at 5' region of HC (Heavy Chain) DNA effect on the amount of product.

PUREfrex® 2.0 + DS supplement

↓ + HC DNA (PCR product) from Herceptin
↓ incubation at 37°C for 4 h
↓ SDS-PAGE (0.5 µL of reaction/lane) and Oriole staining

a) nucleotide sequence of HC DNA

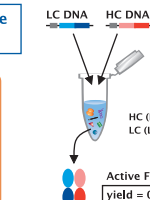
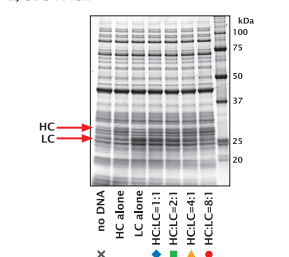
	M	Q	V	L	V	S	G	C	(%)
E. coli freq. codon	atg	cag	gtg	cag	ctg	gtg	gaa	agc	ggc
	gaa								59%
Q-1	atg	cag	gtg	cag	ctg	gtg	gaa	tgg	ggc
Q-2	atg	cag	gtg	cag	ctg	gtg	gaa	tgg	ggc
Q-3	atg	cag	gtg	cag	ctg	gtg	gaa	tgg	ggc
Q-4	atg	cag	gtg	cag	ctg	gtg	gaa	tgg	ggc
Q-5	atg	cag	gtg	cag	ctg	gtg	gaa	tgg	ggc
Q-6	atg	cag	gtg	cag	ctg	gtg	gaa	tgg	ggc
E-1	atg	gaa	gtg	cag	ctg	gtg	gaa	tgg	ggc
E-2	atg	gaa	gtg	cag	ctg	gtg	gaa	tgg	ggc
E-3	atg	gaa	gtg	cag	ctg	gtg	gaa	tgg	ggc
E-4	atg	gaa	gtg	cag	ctg	gtg	gaa	agc	ggc
E-5	atg	gaa	gtg	cag	ctg	gtg	gaa	agc	ggc

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

PUREfrex® 2.0 +/- DS supplement / DnaK Mix

↓ + HC and LC (Light Chain) DNA (PCR product) from Herceptin with each ratio
↓ incubation at 37°C or 30°C for 4 h
↓ a), c) SDS-PAGE (0.5 µL of reaction/lane) and Oriole staining
b), d) ELISA

a) SDS-PAGE

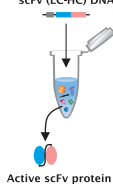


Active scFv can be easily synthesized.

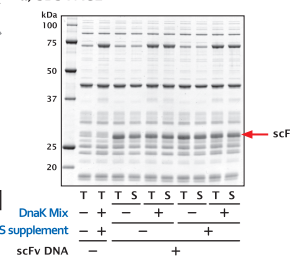
PUREfrex® 2.0 +/- DS supplement / DnaK Mix

↓ + scFv DNA (PCR product) from Herceptin
↓ incubation at 37°C for 4 h
↓ a) ↓ spin at 20,000xg for 30 min)
↓ SDS-PAGE (0.25 µL of reaction/lane) and Oriole staining
b) ELISA

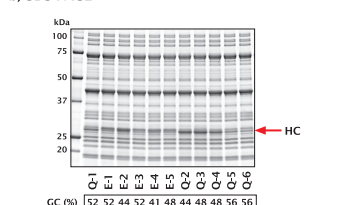
c) SDS-PAGE



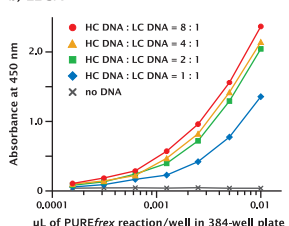
a) SDS-PAGE



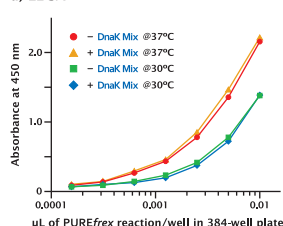
b) SDS-PAGE



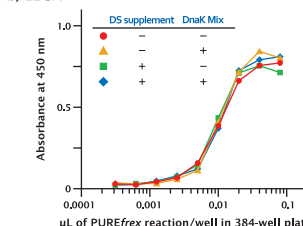
b) ELISA



d) ELISA



b) ELISA



AT rich > GC rich @ 5' region
→ High yield of HC

Optimized H/L DNA ratio
→ High yield of active Fab

All tested conditions
→ High yield of active scFv