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

生産量を増加させた再構成型タンパク質合成系（PURE*frex*® 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い、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PURE*frex*®を完成、製品化した。PURE*frex*®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、PURE*frex*®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PURE*frex*（PURE*frex*® 1.0）比で、単位体積当たり10倍量のGFPを合成できるPURE*frex*® 2.0を完成させることができた。さらにPURE*frex*® 2.0は、PURE*frex*® 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、PURE*frex*® 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。

Summary

E. coli based cell-free protein sysnthesis systems

	Extract system	Reconstituted system		
	S30 system	PURE system (original)	PURE*frex*®1.0	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*®1.0 • PURE*frex*®2.0

a regular kit for the synthesis of proteins without disulfide bonds

DS supplement

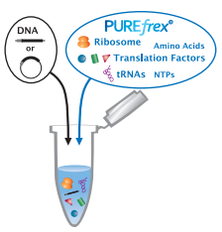
a supplement for the synthesis of proteins containing disulfide bonds

DnaK Mix • GroE Mix

a supplement for the synthesis of aggregate-prone proteins

1. 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

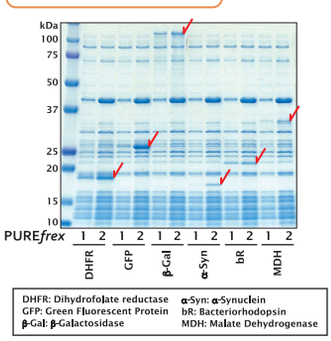
- High throughput preparation of proteins (including Fab, scFv, protein toxin etc.)
- Protein science research
- *in vitro* display
  - Ribosome display
  - mRNA display

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

2. Performance of PURE*frex* 2.0

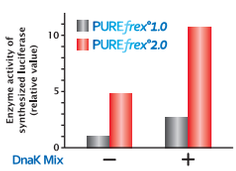
PURE*frex* 2.0 has 2-10 times higher productivity than PURE*frex* 1.0.

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

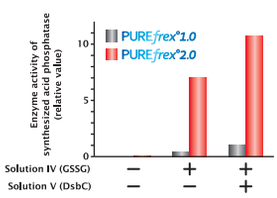


DHFR: Dihydrofolate reductase
GFP: Green Fluorescent Protein
β-Gal: β-Galactosidase
α-Syn: α-Synuclein
bR: Bacteriorhodopsin
MDH: Malate Dehydrogenase

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 and scFv.

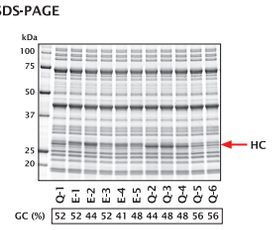
The nucleotide sequence at 5' region of HC (Heavy Chain) DNA effect on the amount of product.

PURE*frex*®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	Q	L	V	E	S	G	GC (%)
E. coli freq. codon		atg	cag	gtg	cag	ctg	gtg	gaa gaa	agc	ggc	63% 59%
Q-1		atg	cag	gtg	caa	ttg	gtc	gaa	tcg	ggt	52%
Q-2		atg	caa	gtt	caa	ttg	gtc	gaa	tcg	ggt	44%
Q-3		atg	cag	gtg	caa	ttg	gtc	gaa	tcg	ggt	48%
Q-4		atg	cag	gtt	caa	ttg	gtc	gaa	tcg	ggt	48%
Q-5		atg	cag	gtg	caa	ctg	gtc	gaa	tcg	ggt	56%
Q-6		atg	cag	gtg	cag	ttg	gtc	gaa	tcg	ggt	56%
E-1		atg	gag	gtg	caa	ttg	gtc	gaa	tcg	ggt	52%
E-2		atg	gaa	gtt	caa	ttg	gtc	gaa	tcg	ggt	44%
E-3		atg	gaa	gtt	cag	ctg	gtc	gaa	tcg	ggt	52%
E-4		atg	gaa	gtt	caa	ttg	gtt	gaa	agc	ggt	41%
E-5		atg	gaa	gtt	cag	ctg	gtt	gaa	agc	ggt	48%

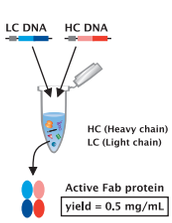
b) SDS-PAGE



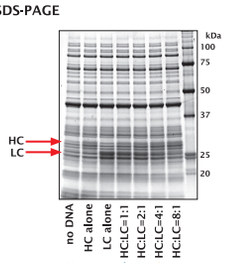
AT rich > GC rich @ 5' region
→ High yield of HC

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

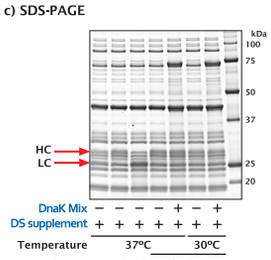
PURE*frex*®2.0 +/- DS supplement / DnaK Mix
↓ + HC and LC (Light Chain) DNA (PCR product) from Herceptin with each ratio
↓ incubation at 37°C or 30°C for 4 h
↓ a), c) SDS-PAGE (0.5 μL of reaction/lane) and Oriole staining
b), d) ELISA



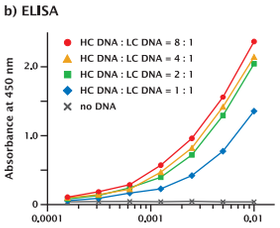
a) SDS-PAGE



c) SDS-PAGE



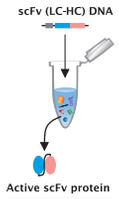
b) ELISA



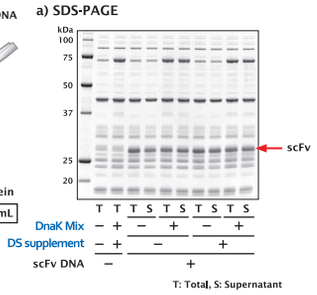
Optimized H/L DNA ratio
→ High yield of active Fab

Active scFv can be easily synthesized.

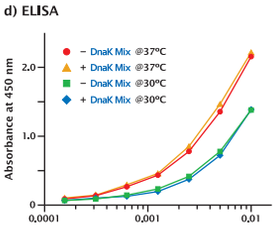
PURE*frex*®2.0 +/- DS supplement / DnaK Mix
↓ + scFv DNA (PCR product) from Herceptin
↓ incubation at 37°C for 4 h
↓ a) ↓ spin at 20,000xg for 30 min)
↓ SDS-PAGE (0.25 μL of reaction/lane) and Oriole staining
b) ELISA



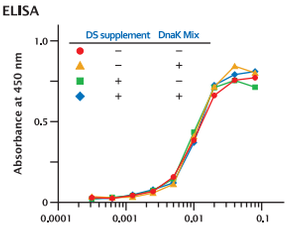
a) SDS-PAGE



d) ELISA



b) ELISA



All tested conditions
→ High yield of active scFv