Unique cell-free protein synthesis system, PURE*frex*
- Useful platform for protein expression in the development of biologics -

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Abstract

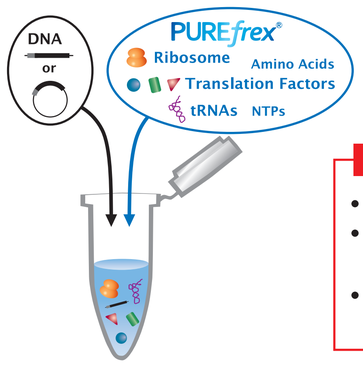
PURE*frex*® is a cell-free protein synthesis system based on the PURE (Protein synthesis Using Recombinant Elements) system. PURE system is a reconstituted cell-free protein synthesis system, which consists of only purified factors necessary for transcription, translation and energy regeneration. The PURE system has the unique features. It contains less contaminant such as nucleases and proteases, and the composition of the reagents can be easily adjusted in accordance with the purpose. We refined the preparation methods of all components that were purified from *E. coli* and developed the new PURE system as "PURE*frex*®". The latest version of PURE*frex*® is PURE*frex*® *2.0*, which has the productivity of GFP and *E. coli* dihydrofolate reductase reaching to approximately 1 mg/mL in simple batch mode. The product is easily purified by simple method, and also it is directly applicable to cell-based assay even without purification because of very low endotoxin level.

Here we report three topics about PURE*frex*® *2.0* and PURE*frex*® *2.1*.

1. AT-rich codon at the N-terminal region of ORF facilitates the productivity in various proteins including proteins containing disulfide bonds and membrane proteins.
   For example, 600 μg/mL of heavy chain of Trastuzumab could be synthesized from the template DNA containing AT-rich codon at the N-terminal region.
2. Reducing reagents in the reaction mixture have influence on the formation of disulfide bonds in the synthesized protein.
   When alkaline phosphatase (AP) was synthesized in the presence of DTT, synthesized AP was not active, while synthesized AP with reduced glutathione (GSH) was active.
3. Functional aglycosylated IgGs such as Trastuzumab and Nivolumab could be synthesized with a productivity of 30-120 μg/mL under the optimized condition.

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 product usable as a temlplate DNA

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

PURE*frex*® *2.0*
Regular kit for the protein synthesis containing DTT as a reducing reagent

PURE*frex*® *2.1*
Regular kit for the protein synthesis capable of selecting a reducing reagent

DS supplement
Supplement for the synthesis of proteins containing disulfide bonds

DnaK Mix / GroE Mix
Supplement for the synthesis of aggregate-prone proteins

E. coli-based cell-free protein sysnthesis system

	Extract system	Reconstituted system	
	S30 system	PURE system (original)	PURE*frex*® *2.0*
Typical Yield (μg/mL)	10-1,000	10-200	10-1,000
Contamination			
RNase	very High	Low	very Low
LPS	very High	High	very Low
Template DNA			
Plasmid DNA	OK	OK	OK
PCR product (short 3'-UTR)	NG	OK	OK
Customization of composition	Difficult	Easy	Easy
Purification of His-tagged product	OK	NG	OK

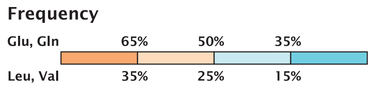
1. N-terminal codon and protein synthesis

Trastuzumab Fab HC

Met-Glu-Val-Gln-Leu-Val-

Glu		Val		Gln		Leu		Val	
codon	Freq. (%)	codon	Freq. (%)	codon	Freq. (%)	codon	Freq. (%)	codon	Freq. (%)
gaa	70	gtt	25	caa	30	tta	15	gtt	25
gag	30	gtc	18	cag	70	ttg	12	gtc	18
		gta	17			ctt	12	gta	17
		gtg	40			ctc	10	gtg	40
						cta	5		
						ctg	46		

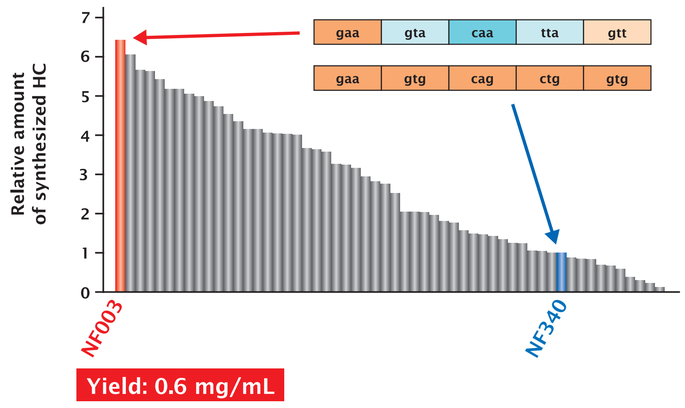
Frequency



All clones: 384
Tested clones: 56

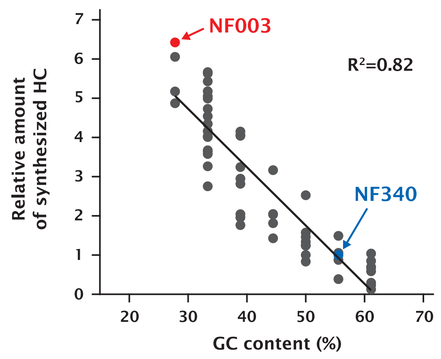
*Frequency is calculated from Codon Usage Database in Kazusa DNA Res. Inst. (*E. coli* K-12 strain)

The amount of product synthesized from the template DNA with different N-terminal codon



Yield: 0.6 mg/mL

Relationship between protein synthesis and GC contents of N-terminal sequence



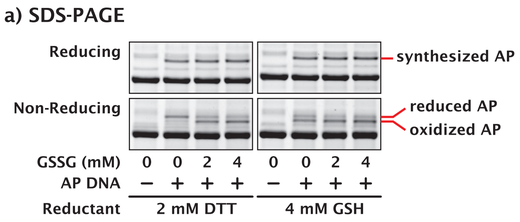
AT-rich codon > Major codon @ 5'-terminus of ORF

2. Synthesis of proteins containing disulfide bonds

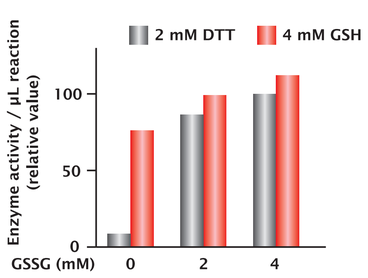
2-1. Alkaline phosphatase (2 disulfide bonds)

PURE*frex*® *2.1* containing DTT or GSH
- ↓ + oxidized glutathione (GSSG) with indicated concentration
- ↓ +/- Alkaline phosphatase (AP) DNA
- ↓ incubation at 37ºC for 4 h
- ↓ a) SDS-PAGE under reducing and non-reducing condition
  b) AP enzymatic activity assay

a) SDS-PAGE

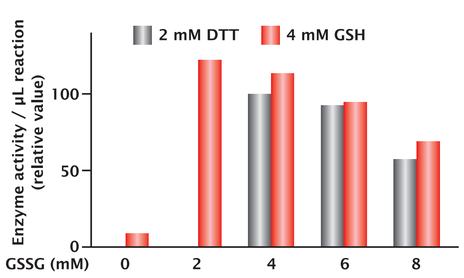


b) Activity assay



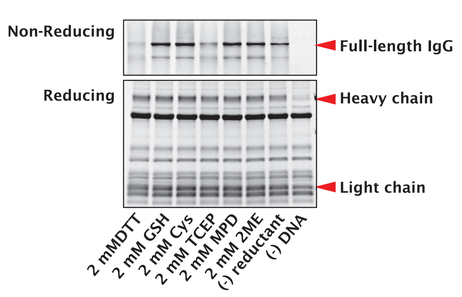
2-2. Acid phosphatase (5 disulfide bonds)

PURE*frex*® *2.1* containing DTT or GSH
- ↓ + GSSG and 4 μM DsbC
- ↓ +/- Acid phosphatase (AppA) DNA
- ↓ incubation at 37ºC for 4 h
- ↓ AppA enzymatic activity assay



2-3. IgG (Trastuzumab) (16 disulfide bonds)

PURE*frex*® *2.1* containing indicated reducing reagent
- ↓ + 3 mM GSSG, 5 μM DsbC and 5 μM DnaK/1 μM DnaJ/1 μM GrpE
- ↓ +/- Trastuzumab HC and LC DNA
- ↓ incubation at 37ºC for 16 h
- ↓ SDS-PAGE under reducing and non-reducing condition



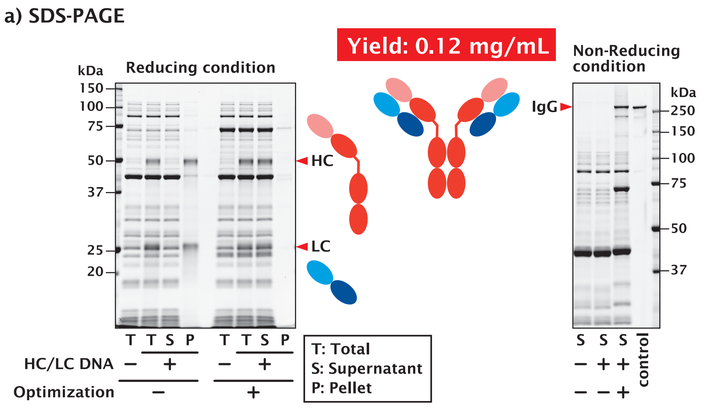
Reducing reagents influence on the formation of correct disulfide bonds within and between synthesized polypeptides.

3. Synthesis of functional aglycosylated IgG

PURE*frex*® *2.1* containing DTT or GSH
- ↓ +/- DS Supplement (GSSG, DsbC) and DnaK Mix (DnaK/DnaJ/GrpE)
- ↓ +/- Trastuzumab HC and LC DNA
- ↓ incubation at 37ºC for 28 h
- ↓ a) SDS-PAGE under reducing and non-reducing condition
  b) ELISA

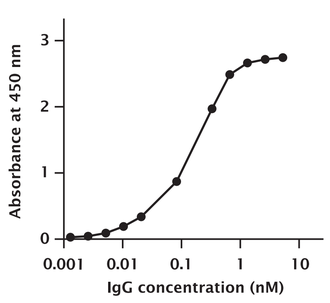
a) SDS-PAGE

Yield: 0.12 mg/mL



Key Factors for IgG synthesis
- HC:LC DNA ratio (4:1-1:2)
- Redox state (+GSH/GSSG)
- Molecular chaperones (+DnaK, +DsbC)
- Incubation Temperature (30-37ºC)
- Incubation Time (>24 hours)

b) ELISA



Summary of synthesis of various IgGs using PURE*frex*

Name	Subclass	Antigen	Synthesis Temp (ºC)	Template DNA Ratio (HC:LC)	Yield (μg/mL)	EC_{50} (nM)
Trastuzumab	IgG1	Her2	37	4:1-2:1	124.4	0.16
Adalimumab	IgG1	TNFα	37	4:1-2:1	46.3	0.1
Cetuximab	IgG1	EGFR	30	1:2	49.2	0.02
Panitumumab	IgG2	EGFR	37	1:2	32.8	0.036
Nivolumab	IgG4	PD1	30	2:1	72.5	0.05

EC_{50}: 50% effective concentration of antigen binding activity by ELISA

Functional aglycosylated IgG can be synthesized using PURE*frex* under the optimized condition.

For more information, please contact us.

URL: www.genefrontier.com/en e-mail: purefrex@genefrontier.com

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