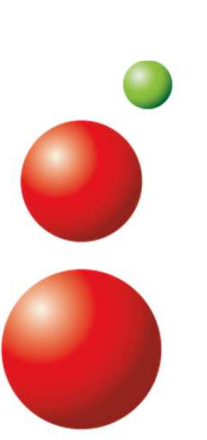


Efficient *in vitro* expression of aglycosylated full-length IgG using a reconstituted cell-free protein synthesis system, PUREfrex® 2.0



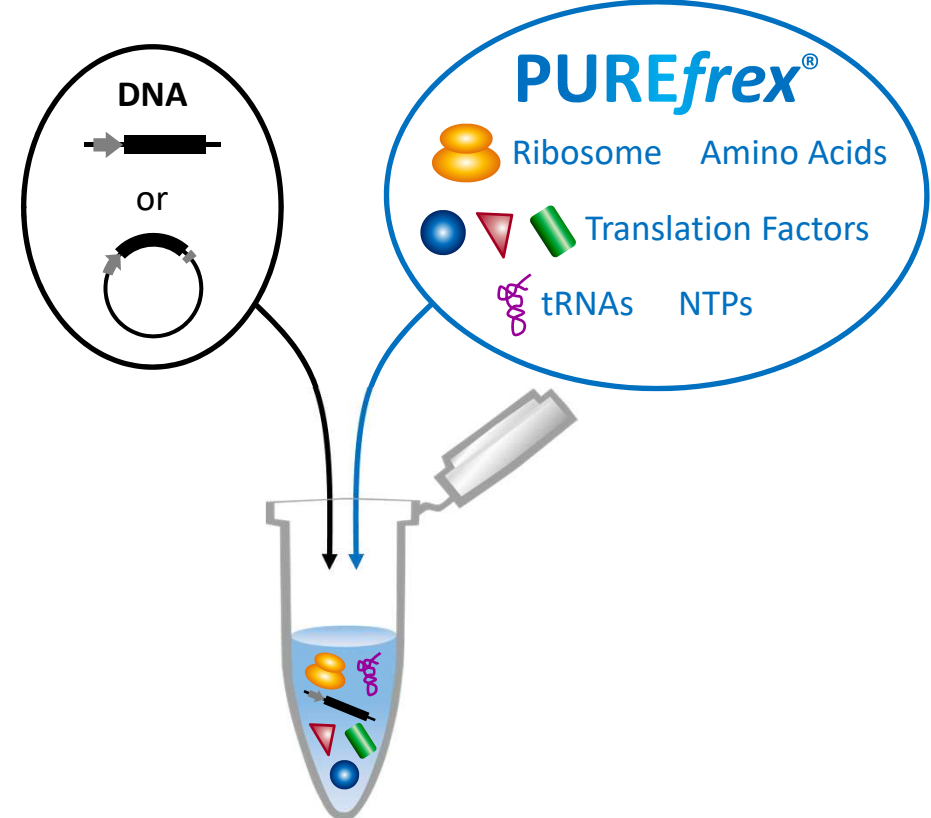
GeneFrontier

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Abstract: Aglycosylated full-length IgG (anti-HER2 monoclonal antibody) was synthesized using PUREfrex 2.0. 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. Recently, we developed an updated PURE system with higher productivity, which was launched as “PUREfrex 2.0” in 2015. We reported that PUREfrex 2.0 could be used for production of functional proteins such as Fab, scFv, a protein based toxin and an immunotoxin last year. Here, we report the further application using PUREfrex 2.0 for production of IgG. To synthesize IgG, we optimized the composition of the reaction mixture and the reaction conditions as below; 1) adding molecular chaperone (DnaK mix) to increase the solubility of the product; 2) adding DsbC to form disulfide bonds between the correct cysteine residues; 3) adjusting GSH/GSSG ratio for optimum redox state; 4) long-time incubation (over 20h) to assemble the hetero tetramer of two heavy chains and two light chains. At the best mode of synthesis, the productivity of IgG reached to 0.042 mg/mL. The synthesized IgG was detected as single band on non-reduced SDS-PAGE after the purification by protein A resin and the following gel filtration. The purified IgG exhibited high binding affinity to recombinant HER2 protein. Fifty percent effective concentration of target binding activity (EC₅₀) in ELISA was 0.16 nM, which is similar to the trastuzumab. This result indicates that PUREfrex 2.0 will be useful tool for high-throughput expression/screening of functional antibodies (scFv, Fab, and IgG).

1. PUREfrex®; based on the PURE system technology

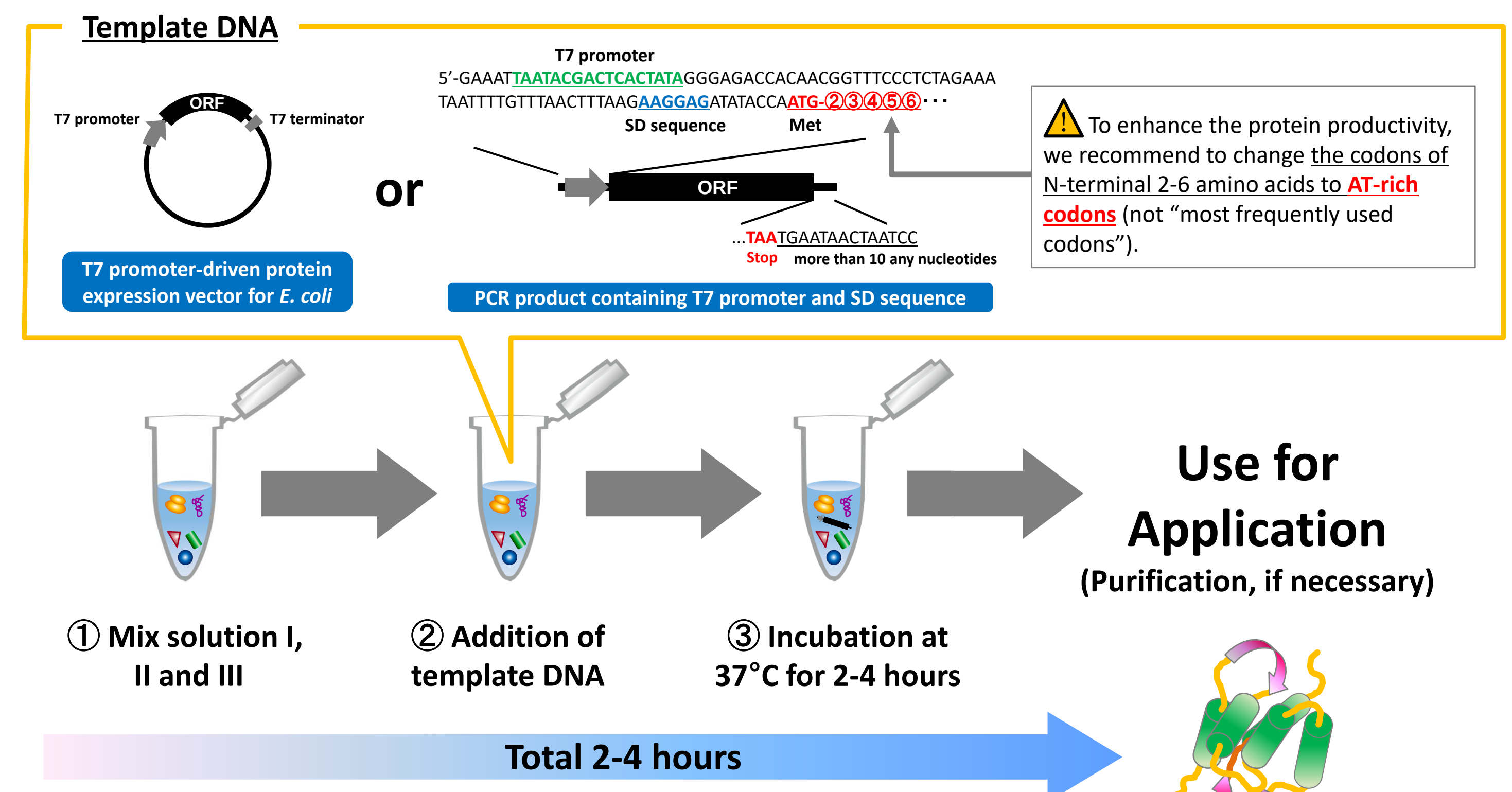


The PURE (Protein synthesis Using Recombinant Elements) system is a reconstituted cell-free protein synthesis system, which consists of **only purified factors** necessary for transcription, translation and energy regeneration.

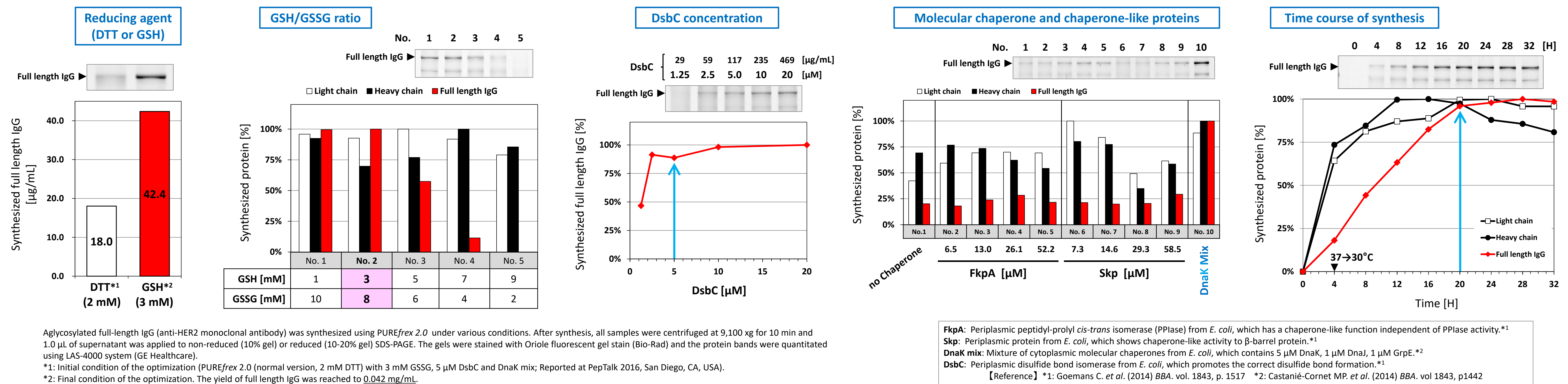
	E. coli based cell-free protein synthesis systems			
	Extract system	Reconstituted system		
	S30 system	PURE system (original*)	PUREfrex® 1.0	PUREfrex® 2.0
Typical Yield (μg/mL)	100-1,000	10-200	10-200	20-1,000
Contamination				
RNase	very High	Low	very Low	very Low
Endotoxin (LPS)	very High	High	very 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
In vitro display				
Ribosome display	△	○	◎	○
mRNA display	△	○	◎	○

【Reference】*: Shimizu Y. et al. (2001) Nat. Biotechnol., vol. 19, p. 751

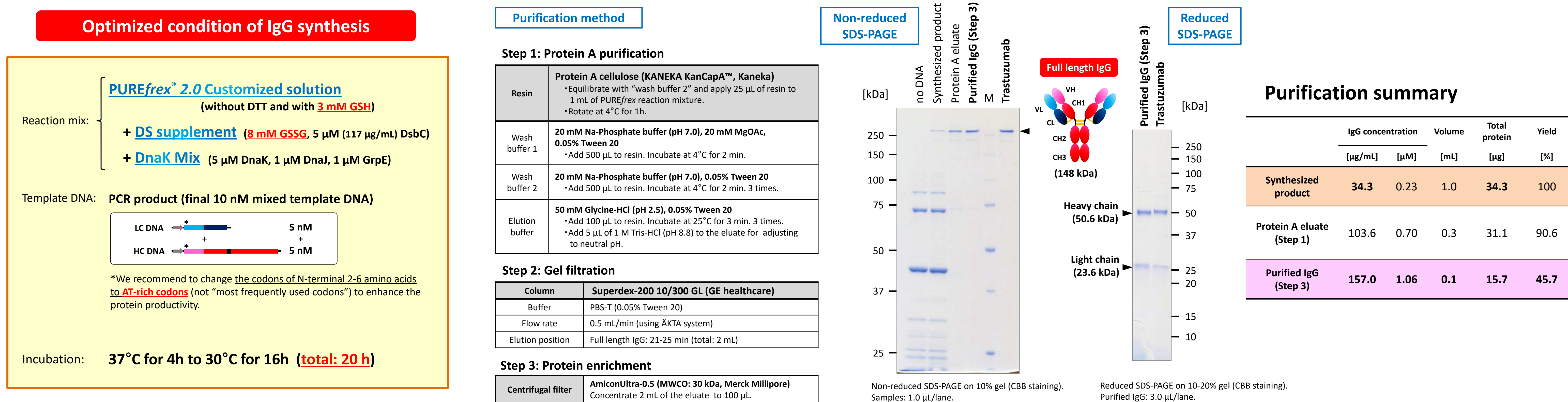
2. How to use PUREfrex®; “Easy” and “High throughput” preparation of Proteins



3. Optimization of the synthesis of full length IgG

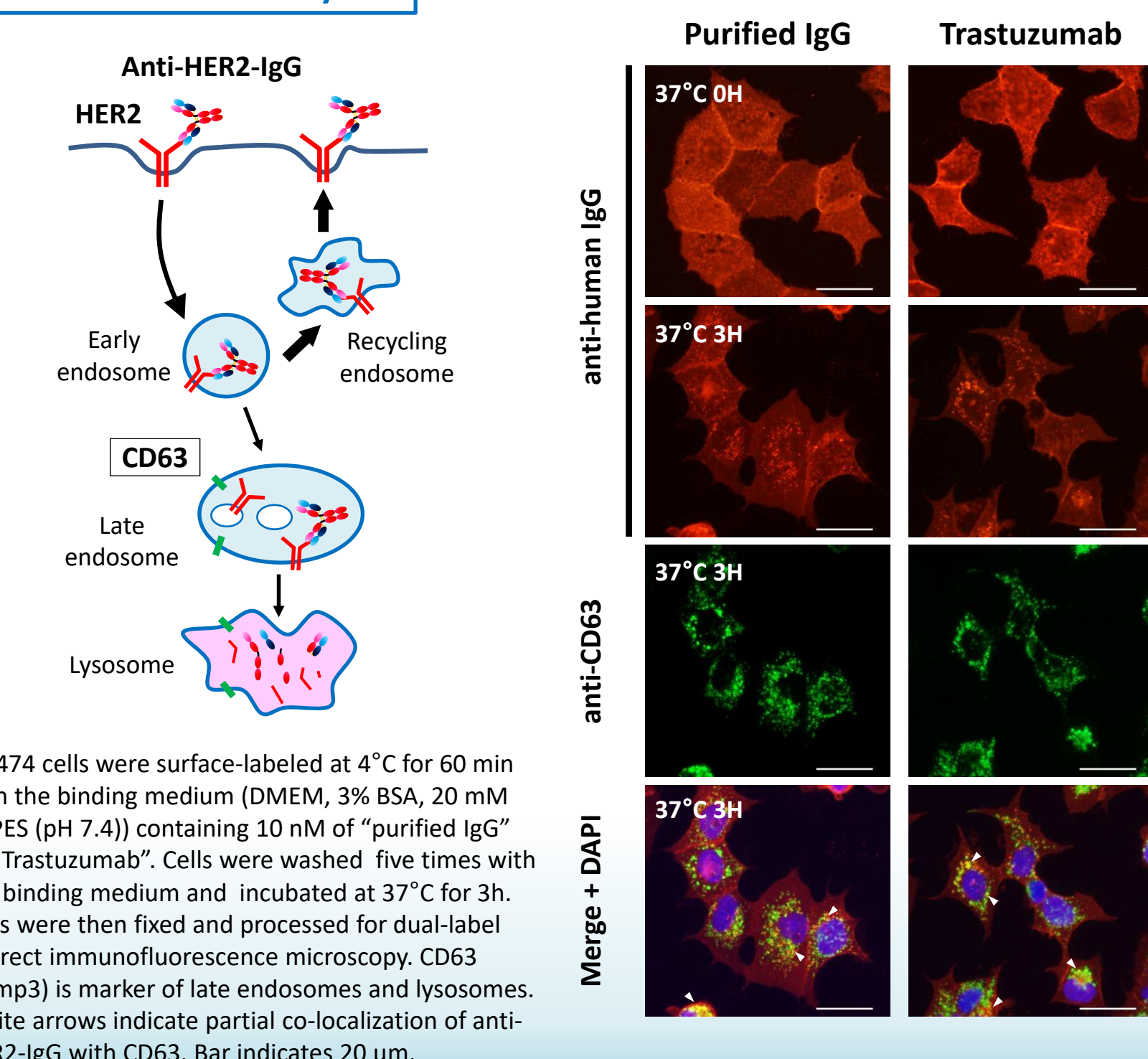


4. Synthesis and purification of full length IgG using PUREfrex® 2.0

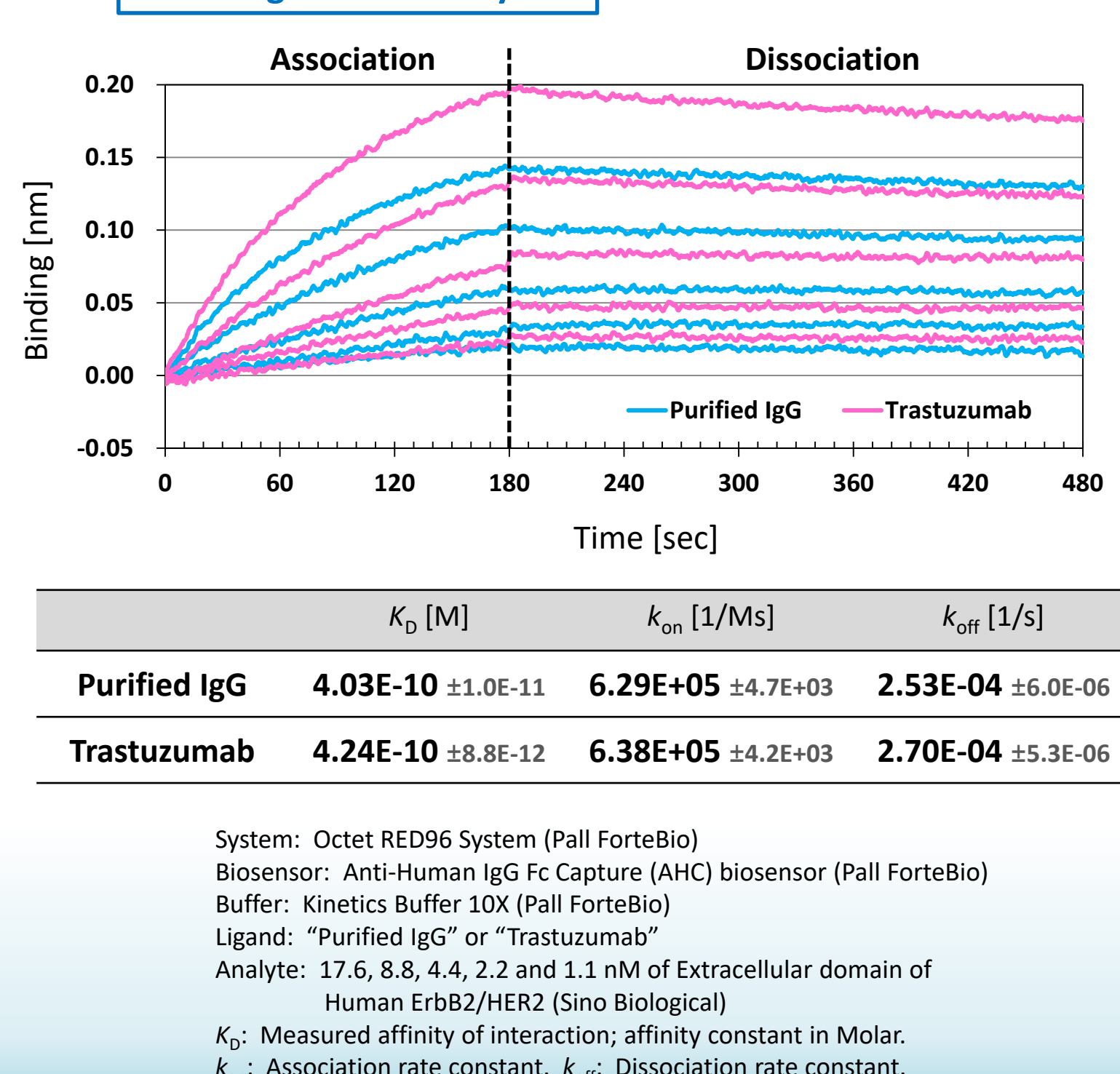


5. Evaluation of purified full length IgG

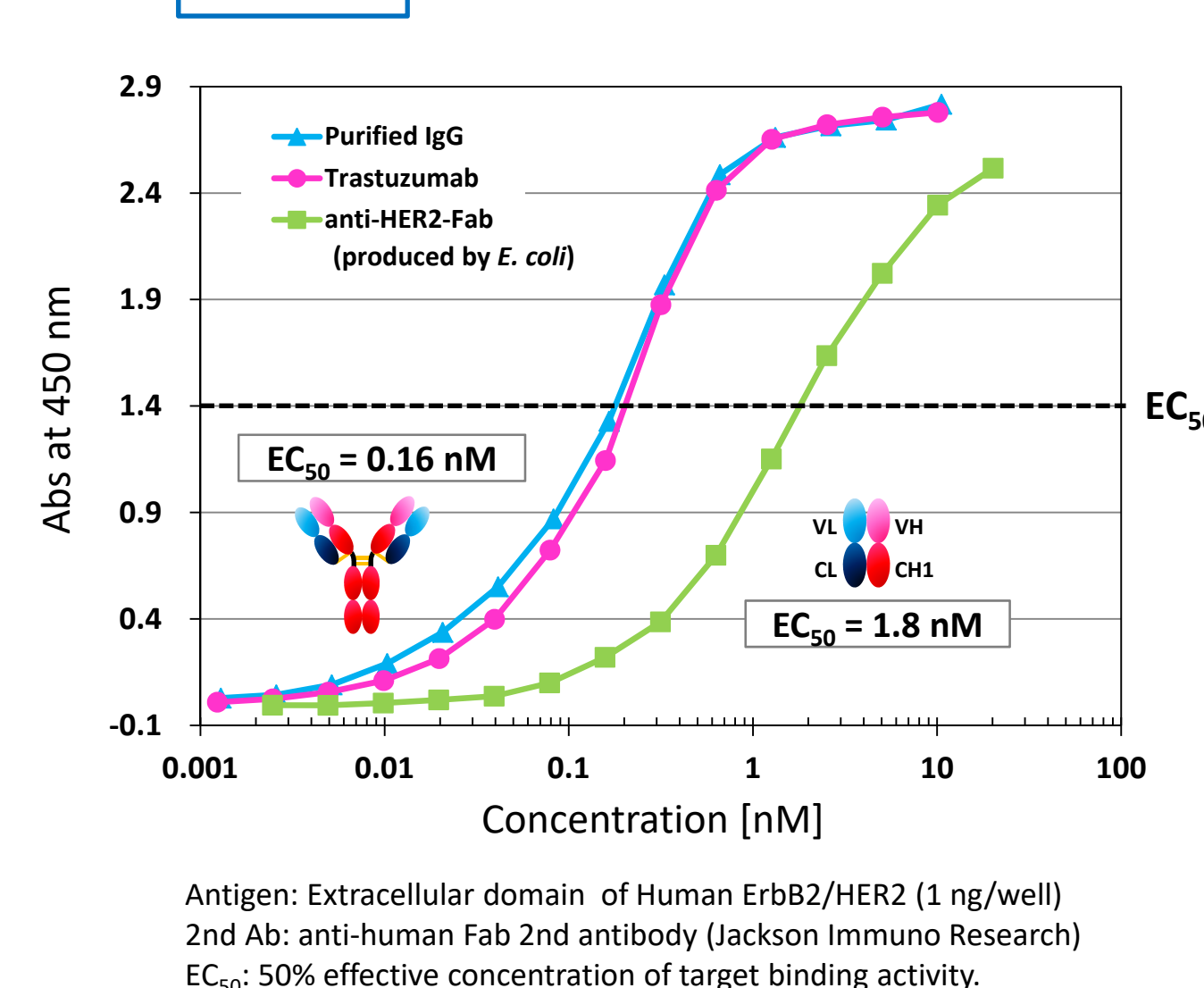
Internalization analysis



Binding kinetics analysis

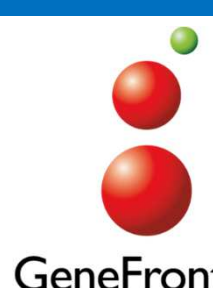


ELISA



Conclusion

- Aglycosylated full-length IgG was synthesized in the active form using PUREfrex 2.0.
 - Redox state (GSH/GSSG ratio) and long-time incubation (over 20h) were important to form a correct hetero tetramer of two heavy chains and two light chains.
 - The 15.7 μg of synthesized IgG was purified from 1.0 mL of PUREfrex 2.0 reaction mixture.
- ### On-going Project
- Synthesis of other IgGs. (e.g. anti-TNF-α, EGFR, VEGF, etc.)
 - Synthesis of bispecific IgG antibody.
- ### Acknowledgements
- We thank Dr. Yosuke Uchinashi (Nihon Pall Ltd.) and Keita Iguchi (KANEKA Corporation) for technical support and experimental advice.



Our Products:

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