

Application Note

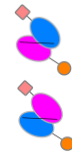
Trastuzumab (anti-HER2) ScFv and Fab

(a) Constructs

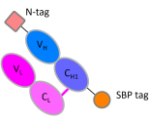
ScFv_HL



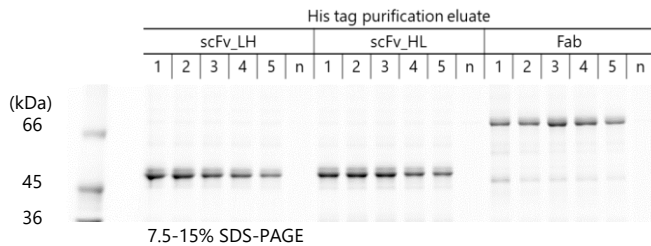
ScFv_LH



Fab (coexpression)



(b) Cell-free protein synthesis



CFPS : 30-1000 μ L, 30°C, o/n

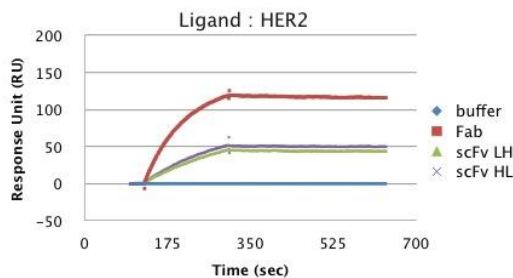
Redox Condition

1. 5 mM GSH
2. 4 mM GSH / 1 mM GSSG
3. 2.5 mM GSH / 2.5 mM GSSG
4. 1 mM GSH / 4 mM GSSG
5. 5 mM GSSG
- n. No DNA

(c) SPR analysis (Biacore3000)

Ligand : HER2/CM5 (amine coupled)

Buffer : HBS-EP, 30 μ L/min

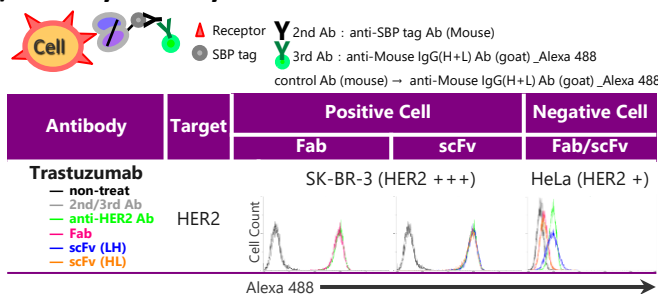


Antibody	Tar get	Ligand : AFs			Ligand : HER2		
		k_{on}	k_{off}	K_D (nM)	k_{on}	k_{off}	K_D (nM)
Trastuzumab	scFv	4.01×10^5	9.42×10^{-4}	2.35	6.21×10^5	5.89×10^{-5}	0.10
anti-HER2	Fab	5.74×10^5	1.13×10^{-3}	1.97	1.03×10^6	1.55×10^{-4}	0.15

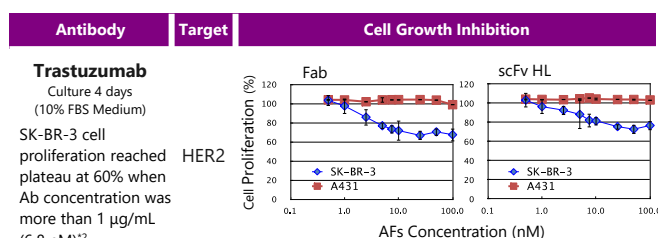
$K_D = 5$ nM ^{††}(Cell based assay)

*1) Drugs@FDA, Herceptin label (1998/09/25)

(e) Flow cytometry



(f) Neutralization analysis

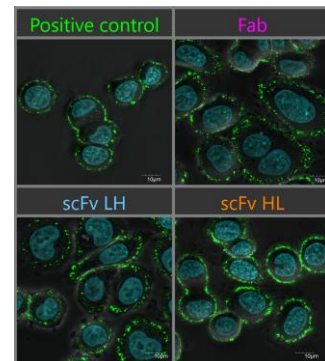


*2) Tokuda Y, et al., British Journal of Cancer 73:1362-1365,1996

(d) Confocal microscopy (SK-BK-3 : HER2+++)

Green : Alexa488 (AFs)

Blue : Hoechst33342



Summary

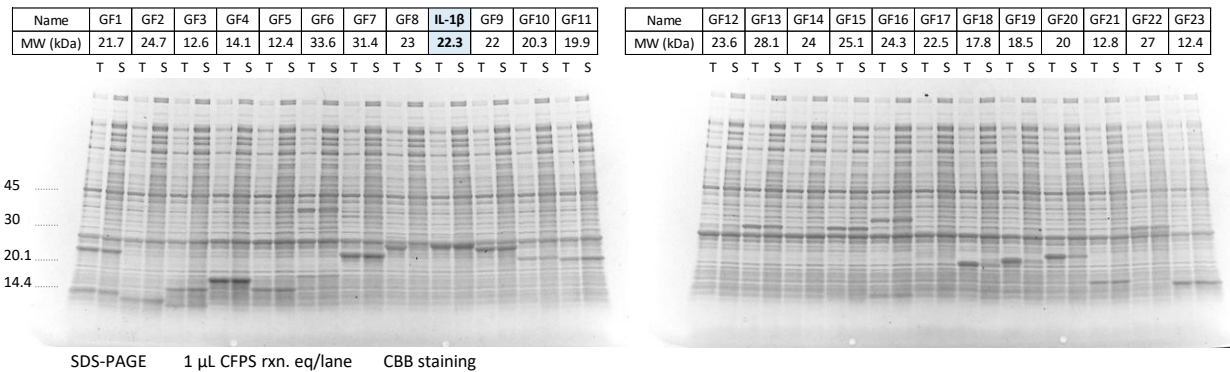
ScFv and Fab forms of Trastuzumab were synthesized by cell-free protein synthesis and evaluated.

- (a) Linear template DNAs for scFv_HL, scFv_LH, H and L were prepared.
- (b) Cell-free protein synthesis was carried out under different redox conditions. Optimal redox conditions varied somewhat by construct. For Fab synthesis, H and L templates were co-expressed.
- (c) Affinity to the HER2 ligand was evaluated by Biacore. KDs in the subnanomolar to nanomolar range were observed. The KD value for Ab was referenced from Drug@FDA Herceptin label.
- (d) Affinity to HER2 positive cells was evaluated by staining with Alexa488-labeled Fab, scFV_LH and scFV_HL affinity fragments (AFs). The AFs showed staining patterns similar to the positive control sample (Alexa488-labeled Ab).
- (e) Affinity to HER2 positive cells was evaluated by flow cytometry. All the AFs and Ab showed the same fluorescent yield for the positive cells.
- (f) Functional assay for Fab and scFv_HL was conducted. Fab and scFv_HL inhibited SK-BR-3 cell growth at similar extent as Trastuzumab. The result indicated that the Fab and scFv_HL were functionally active.

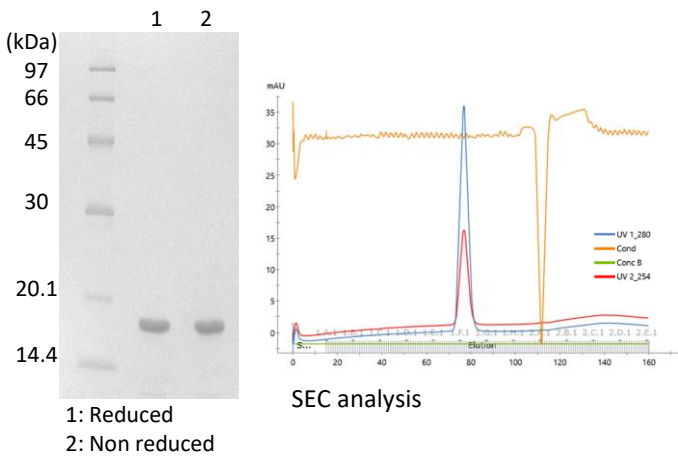
Data provided by Dr. Kigawa, RIKEN

Cell growth factors and cytokines

(a) Expression board



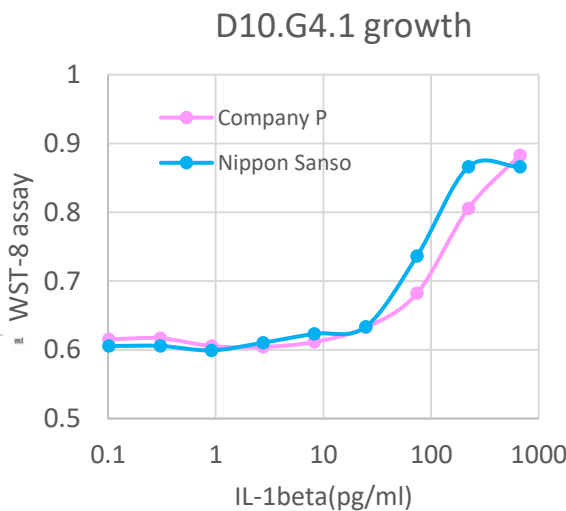
(b) IL-1 β purification



5 mL CFPS → 1.14 mg purified IL-1 β

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(c) IL-1 β evaluation



Summary

Various cell growth factors and cytokines were synthesized by CFPS. IL-1 β purification and evaluation results are shown as an example.

- (a) A part of our cell growth factors and cytokines expression board by CFPS kit (Musaibo Kun N SS kit, A239-0304).
- (b) IL-1 β was expressed on semi-preparative scale CFPS (5 mL) and purified via 1st His-affinity, tag cleavage, 2nd His-affinity, IEX, and SEC. Monodispersed IL-1 β (1.14 mg) was successfully purified.
- (c) The activity of IL-1 β was evaluated by the D10.G4.1 cell proliferation. Cell concentration was assessed by the WST-8 assay. Our IL-1 β showed similar specific activity to a commercially available product.

