Simple method for screening and production of antibody using *ExiProgen*™

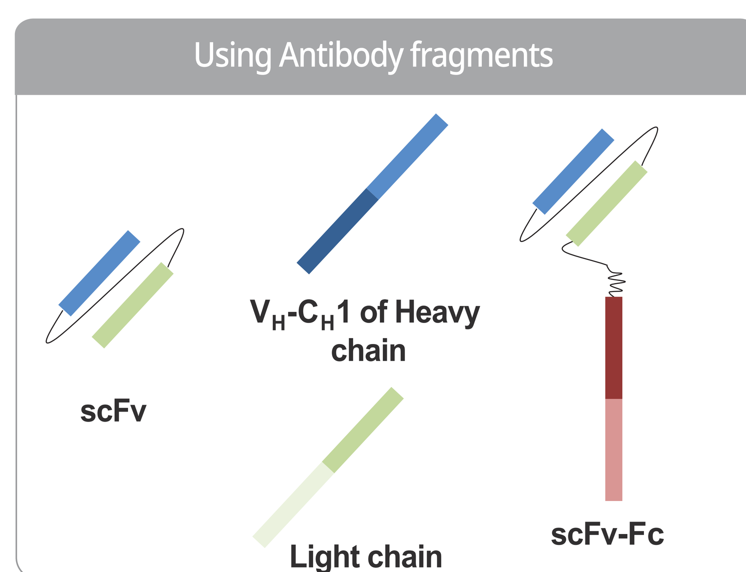
Abstract

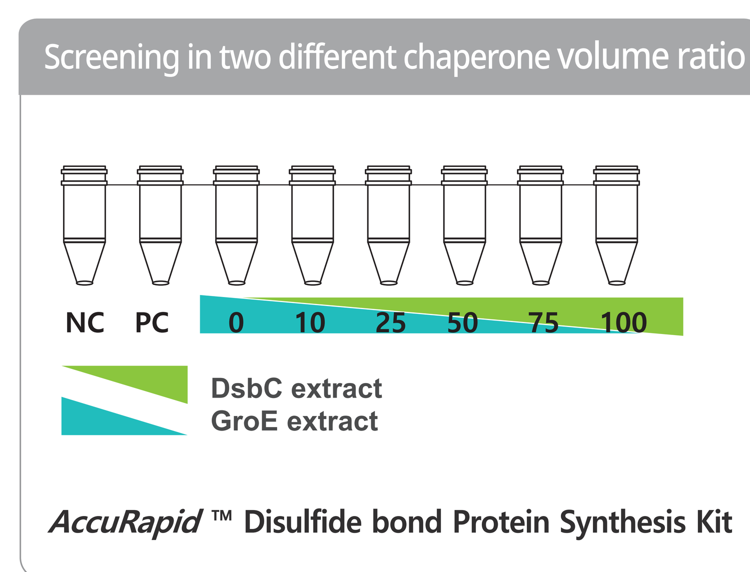
ExiProgen™is a novel and innovative machine that combines cell-free protein synthesis and affinity purification automatically. Bioneer provides various protein synthesis kits that have been optimized for many different proteins including disulfide-bonded protein.
Antibody and its derivatives play great roles in immunology as well as in pharmaceutics. Therefore screening, selection and production of well-designed those materials have significant importance.

scFv, scFv-Fc antibodies and other derivatives were designed then evaluated in its expression/purification. The best ratio of molecular chaperone proteins for disulfide bond formation was also determined for each antibody/antibody derivatives. Additionally, *ExiProgen*™ Bulk Kit was designed to produce 10-fold larger amount of scFv-Fc or other antibody derivatives than *ExiProgen*™ EC-Disulfide Protein Synthesis Kit. Therefore, it is concluded that *ExiProgen*™ would be a valuable tool for screening and production of antibody and its derivatives.

Materials and Methods

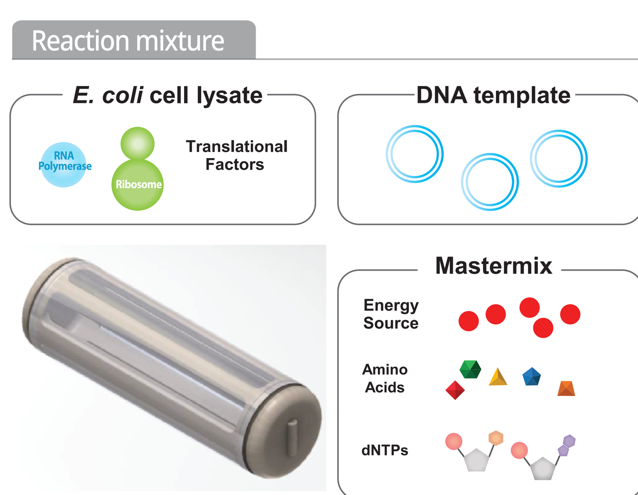
Four genes, scFv, light chain, VH-CH1 of Heavy chain of antibody and scFv-Fc fusion protein were amplified through PCR and inserted into pET23 vector. To optimize for expression antibody fragments were carried out screening with two chaperones - DsbC and GroE extract in 0-100% in volume ratio. At the initial screening *AccuRapid*™ Disulfide bond Protein Synthesis Kit was used for manual protein expression.





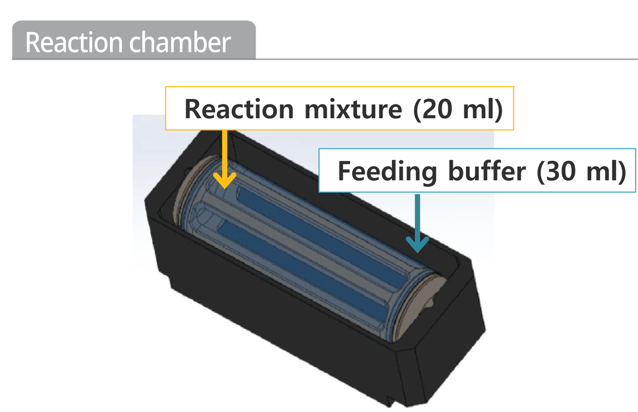
Brief scheme of Bulk Kit

Reaction mixture



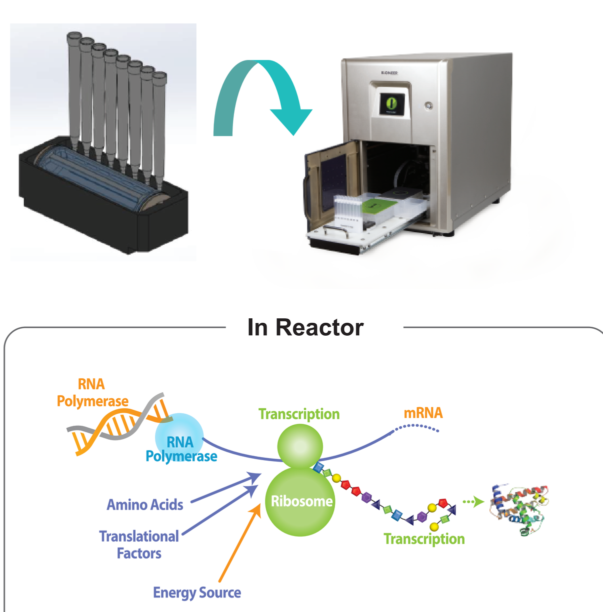
After initial screening, protein expression were carried out in Bulk kit system which consisted of two cartridges, cylindrical reactor, and reaction chamber. The reactor was filled with about 20 mL of reaction mixture and covered with semi-membrane at external region.

Reaction chamber



Reactor, filled with reaction mixture including PCR products or plasmid DNA as template, was installed at reaction chamber. Then reaction chamber was filled with 30 mL of feeding buffer.

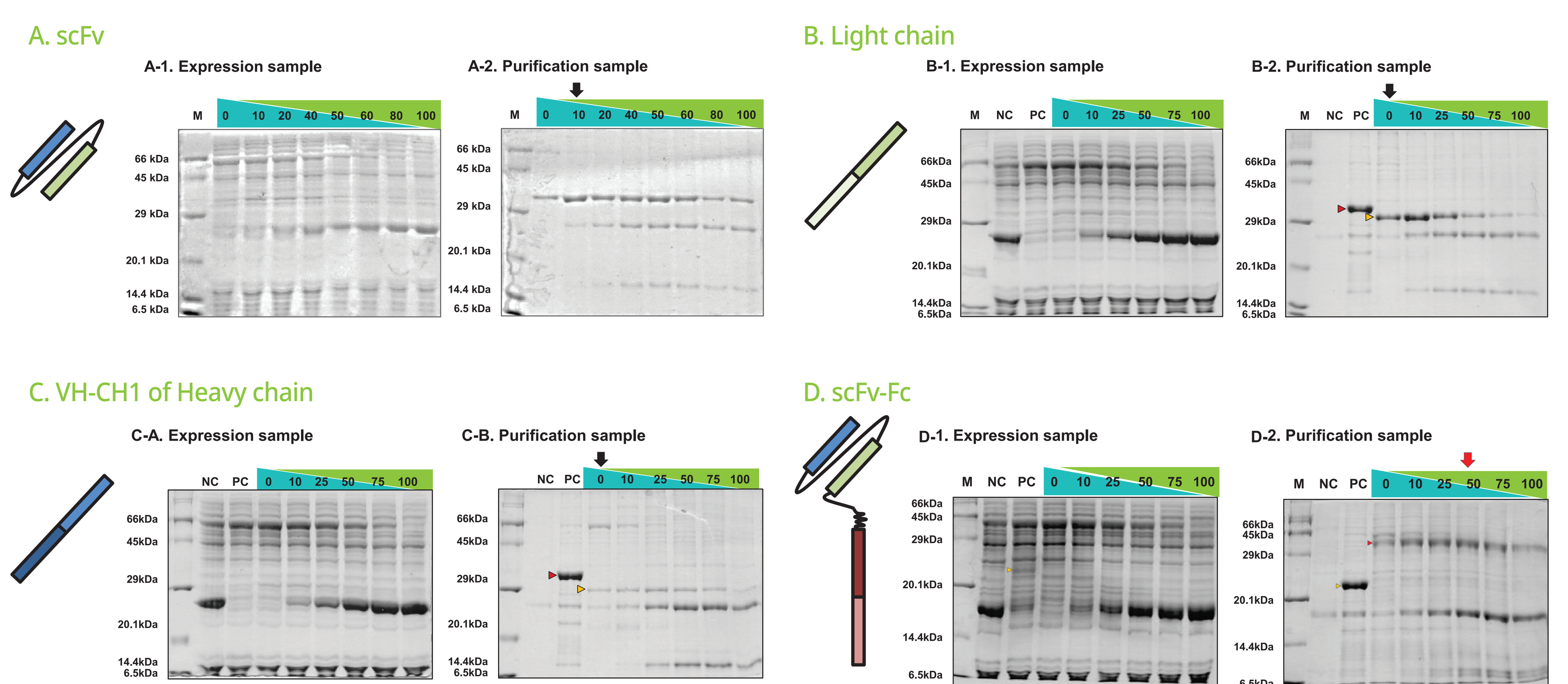
SECF using *ExiProgen*™



After kit setup on *ExiProgen*™ protein expression reaction was started under pre-installed protocol. During the reaction extra volume of feeding buffer was supplied 12 times into reaction chamber to increase protein expression according to SECF (Stepwise Exchange Cell-Free) mode.

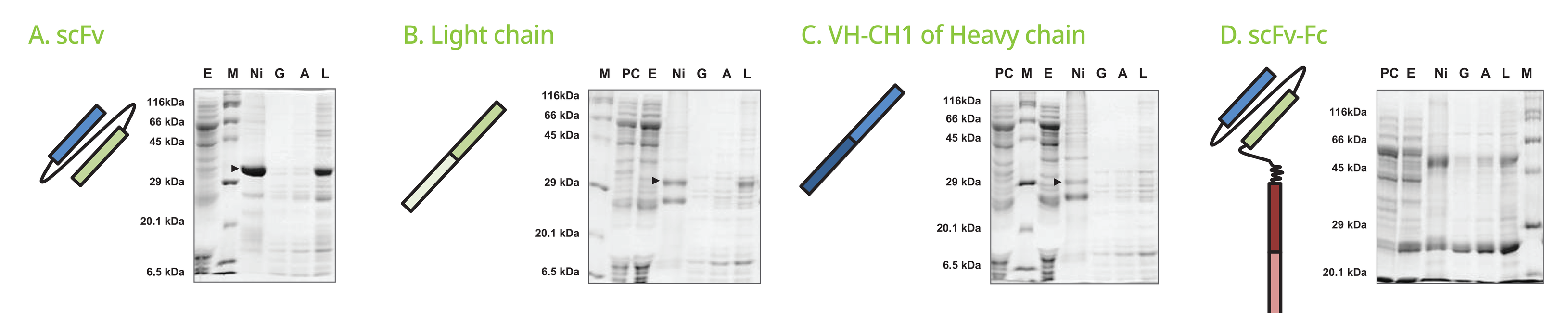
Result

Screening of DsbC/GroE ratio for optimal production for antibody fragments.



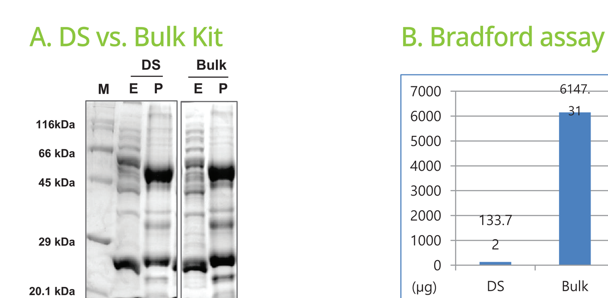
12% SDS PAGE gel stained with Coomassie Brilliant Blue of (A) anti-HA scFv, (B) Light chain, (C) VH-CH1 of Heavy chain, (D) scFv-Fc, respectively. Antibody fragments were expressed using *AccuRapid*™ Disulfide bond Protein Synthesis Kit at 30°C for 3 hours. Reaction volumes is 750 ul. PCR product are used 670 ng/ml, respectively. Orange and light blue triangles show DsbC and GroE extract concentration, respectively. M; protein size marker (Bioneer), NC1; negative control (w/o DNA, GroE 100% extract), NC2; negative control (w/o DNA, DsbC 100% extract), 0~100: ration of DsbC extract in total extract.

Automatic purification various antibody with Protein A, Protein G & Protein L beads.



12% SDS PAGE gel stained with Coomassie Brilliant Blue of (A) anti-HA scFv, (B) Light chain, (C) VH-CH1 of Heavy chain, (D) scFv-Fc, respectively. Antibody fragments were expressed using *AccuRapid*™ Disulfide bond Protein Synthesis Kit at 30°C for 3 hours. Reaction volumes is 750ul. PCR product are used 670ng/ml, respectively. Total elution volume from Ni-NTA beads and Protein G, Protein A, Protein L beads are 120 μl, respectively. Purification were using *ExiProgen*™ with Ni-NTA magnetic bead (10mg), Protein G magnetic bead (10 mg), Protein A magnetic bead (10mg), Protein L magnetic bead (10 mg). M; protein size marker (Bioneer), E; expression sample, Ni; purification sample of Ni-NTA magnetic bead, G; purification sample of Protein G bead, purification sample of Protein A bead, L; purification sample of Protein L bead. 2-Me; β-mercaptoethanol. Amount of loading purification sample is 1/13 of total sample.

Production comparison between previous Kit and new Bulk Kit



A shows 12% SDS PAGE gel stained with Coomassie Brilliant Blue of ExiProgen EC-Disulfide Protein Synthesis Kit (DS) and ExiProgen Bulk Kit (Bulk). B is the data of Bradford assay from both proteins purified. E and P lane of DS are loaded with expression sample of 1/360 of total sample, purification sample of 1/13 of total sample, respectively. E and P lane of Bulk are loaded with expression sample of 1/16K of total sample, purification sample of 1/587 of total sample, respectively.

Summary

- Screening of chaperone proteins was performed to determine the best value in their ratio for antibody fragment expression.
- New device for higher yield of protein production in *ExiProgen*™ was designed. This device was able to harvest about 40-fold increased amount of protein compared to current method.
- Antibody fragments were expressed then purified in *ExiProgen*™ with various magnetic beads - Protein G, A or L - according to their regions.