Biacore™ 8K+ surface plasmon resonance system as a tool in cell line development

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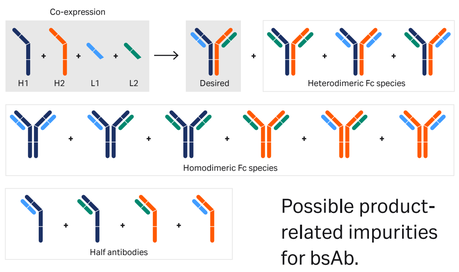
Introduction

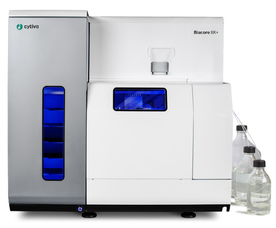
High-throughput titer measurement is important for an efficient cell line development (CLD) process. For titer selection early in the workflow, the chosen technology must allow low sample consumption and high sensitivity. Generation of bispecific antibody (bsAb) producing cell lines can be challenging, since large numbers of clones might have to be screened to find high-producing clones with the correct bsAb composition.

Biacore™ 8K+ surface plasmon resonance (SPR) system can be a valuable tool in the CLD processes. The Biacore 8K+ instrument together with Sensor Chip Protein A offers a ready-to-use solution for CLD of standard mAbs for unattended titer assessment of eleven 96-well plates in 8 to 10 h with a dynamic range of ~ 0.05 mg/L to 10 mg/L. For bsAbs, assessing both the level and purity of the correct bispecific form is critical to identify high-performing producer clones. The protein A (ProtA) titer screening assay can be complemented with molecule specific sandwich assay to quantitate the correct bsAb in the presence of product-related impurities. By combining a bispecific sandwich assay with a ProtA assay, bsAb purity can be obtained and used for clone selection.

Our aim here is to demonstrate how Biacore 8K+ system is used in a CLD process of bsAbs, with screening at the static and deep-well suspension culture stages. ProtA and sandwich assays were used to run multiple plates with CHO clones at different stages to select high-performing clones. Performance of selected clones was further assessed in shake flask fed-batch cultures.



Possible product-related impurities for bsAb.



Biacore 8K+ SPR system.

Materials and methods

We performed CLD work using site-directed integration in a CHO-K1 cell line. Titer screening of multiple plates of CHO cells was undertaken using Biacore 8K+ system at several steps of the CLD workflow, to select for high-performing clones.

In the static plate phase, total Ab titer was measured by ProtA binding (illustrated below). In the shaken deep-well plate phase, an additional sandwich assay with CD3 and a protein L-like peptide binding was used to detect the correct forms of the bsAb (illustrated below). The sandwich assay is described in more detail in poster, “High-throughput Biacore assays for screening and characterization of bispecific antibodies”.

We further evaluated selected clones in shake flask fed-batch cultures, where titer was measured by ProtA and sandwich assays on Biacore 8K+ system. Confirmation of total Ab titer was demonstrated by IgG measurement using the Cedex-Bio analyzer and bsAb pairing was confirmed by LC-MS. Purification of the fed batch material by MabSelect™ VL was undertaken to verify the composition of the bsAb by HPLC.

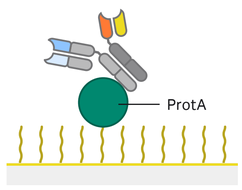


Illustration of antibody binding to protein A on sensor chip.

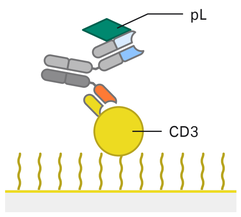


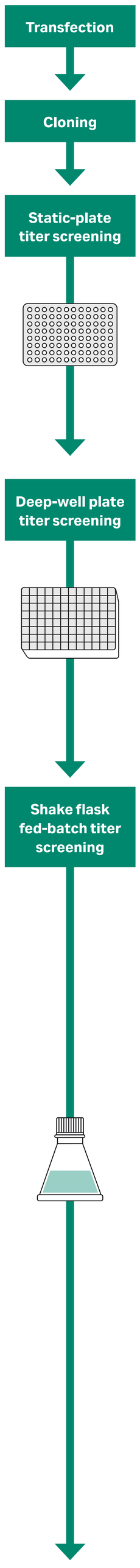
Illustration of sandwich assay. CD3 is immobilized on sensor chip. After bsAb binding, a protein L-like peptide (pL) is flowed over the chip and binds to the correctly paired bsAb.

Conclusions

- Biacore 8K+ system is a valuable tool to identify desired production clones in the CLD workflow. The technology offers novel opportunities for early characterization and selection of bsAb-producer clones.
- We demonstrate how Biacore 8K+ system is used to measure Ab production in multiple plates of CHO clones during a CLD process. Total amount of Ab and correct forms of bsAb production was measured at several steps of the CLD workflow to successfully identify and select for the final production clones.
- Biacore SPR data were in agreement with LC-MS and purification data and could be used as a single method to choose clones for further development.

Product	Product code
Biacore 8K+ SPR system	29722783
Series S Sensor Chip Protein A	29127555
Series S Sensor Chip CM5	29104988
HiTrap™ MabSelect VL resin	17542051
PreDictor™ MabSelect PrismA™ filter plate	17549832

Results



We successfully used a Biacore 8K+ system ProtA assay (Fig 1–3) and sandwich assay (Fig 2–3) in several titer screening steps of the CLD process to narrow down the number of clones and select for a clone with the correct composition for bsAb expression. We used the ratio of correct form of bsAb:total Ab expression to select for the high-performing producer clones. The composition of the bsAb produced by the final clones was confirmed by LC-MS.

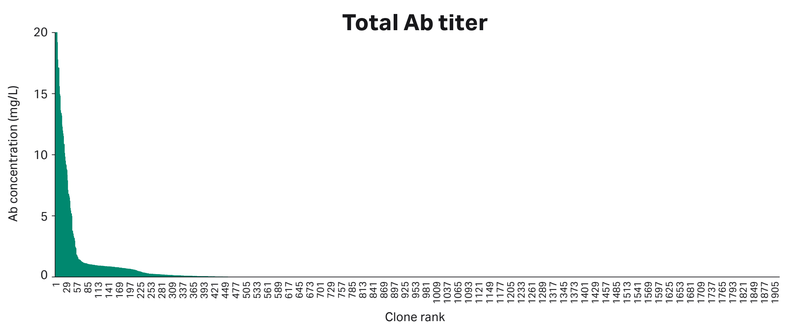


Fig 1. First titer screening from 20 static phase 96-well plates, measuring total Ab titer by protein A binding on Biacore 8K+ system.

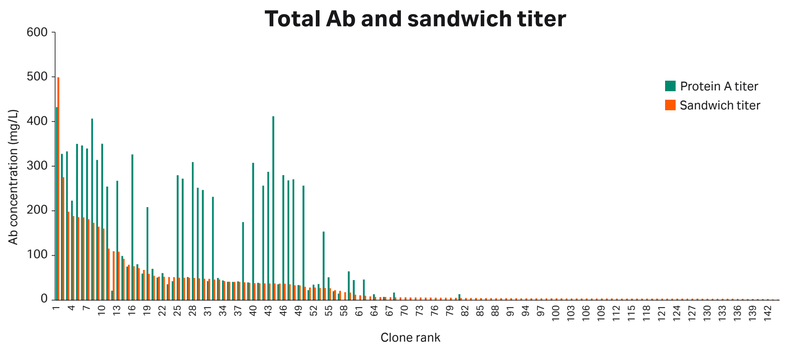


Fig 2. Titer screening from shaken 96-deep well plates, measuring total Ab titer (protein A binding) and correct form of bsAb (CD3 and protein L-like peptide sandwich assay) on Biacore 8K+ system.

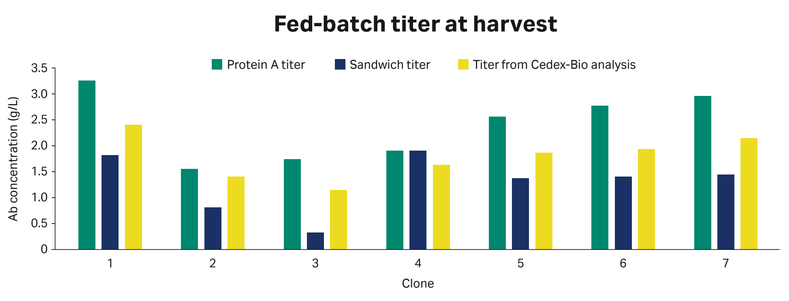


Fig 3. Titer screening of seven clones selected for fed batch in shake flasks. Analysis using Protein A binding for total Ab titer and sandwich assay for correct bsAb assembly titer (CD3 and protein L-like peptide sandwich assay) measured on Biacore 8K+ system. Total Ab titer was also measured with Cedex-Bio IgG assay.

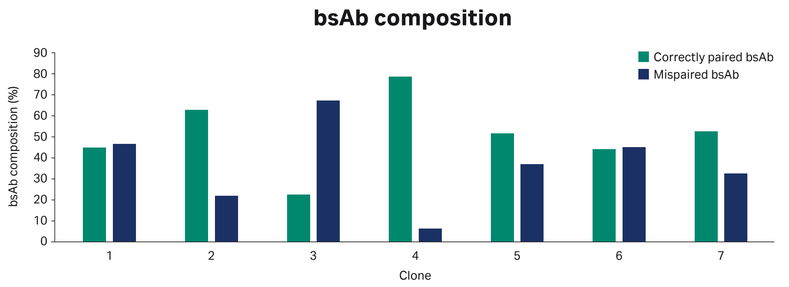


Fig 4. The seven clones were purified with PreDictor MabSelect PrismA plates. Confirmation of amount of correctly paired and mispaired bsAb by LC-MS with UV absorbance at 210 nm. Minor bsAb molecular species are not shown here.

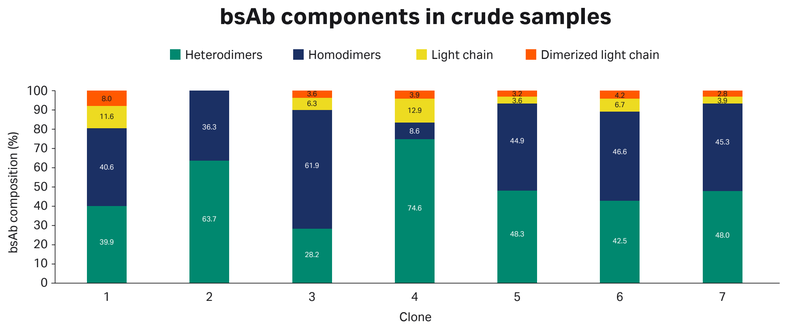


Fig 5. HPLC analysis (percent area at 210 nm) of samples after MabSelect VL purification verifies bsAb composition of the seven clones.

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