Dual parameter screening — two for the price of one

Cynthia Shuman, Anna Moberg, and Linnea Nygren-Babol
Cytiva, Björkgatan 30, 751 84 Uppsala, Sweden

Introduction

The goal of antibody production technology is to obtain high titers of highly specific high-affinity antibodies. To determine whether molecules can successfully progress from discovery to development, scientists evaluate their physicochemical properties in multiple selection rounds. It's been shown that antibody titer correlates with the overall thermostability of molecules, and can refer more specifically to the effective potency of a given antibody sample (1, 2).

Selecting the best antibody molecule based on biological function, efficacy, safety, and developability helps support a more streamlined and successful drug development process.

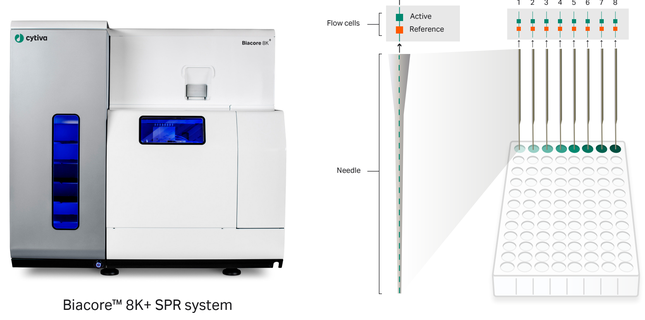
In this poster we present a way to increase analytical efficiency and gain more information from a single analysis. We'll demonstrate the principle using functionality in Biacore™ Insight Evaluation Software to extract antibody titer and kinetics from the same data set in a single run.

Materials and methods

We demonstrated the principle of dual parameter screening using a Mouse Antibody Capture Kit and Biacore™ 8K+ SPR system. Evaluation was performed using Biacore™ Insight Evaluation Software.

We amine-coupled rabbit anti-mouse antibody in 16 flow cells on Series S Sensor Chip CM5. Immobilization levels were approximately 10 000 RU.

We used a mouse monoclonal antibody as ligand and a 12 kDa protein as analyte. We performed regeneration using the reagent supplied in the capture kit, 10 mM Glycine-HCl pH 1.7.



Biacore™ 8K+ SPR system

Fig 1. Biacore™ 8K+ SPR system is equipped with eight channels with two flow cells in each channel. This enables parallel measurement of antibody titer and kinetics for 96 samples in 6.5 h, including time for sample prep.

Principle dual parameter screening

A dual parameter screening experiment is set up as a direct immuno-assay.

1. Attach an antibody capture molecule in reference and active flow cells.
2. Inject antibody in active flow cell. Antibodies bound to capture molecule are used to determine antibody titer.
3. Inject analyte over reference and active flow cells to determine antibody binding kinetics.

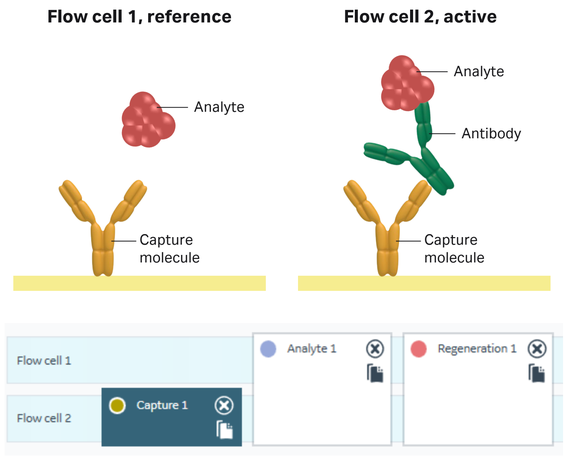


Fig 2. Setup of dual parameter screening. Antibody titer is obtained by assessing binding to a capture molecule. Antibody kinetics is obtained by assessing binding of analyte to the antibody.

Assay setup

Antibody titer is assessed using a parallel concentration analysis setup with one calibration point per channel. We used a channel normalization to compensate for minor channel-to-channel variations. The normalization injection can be the same as one of the calibration solutions.

We assessed antibody kinetics from single-concentration injections of the analyte followed by a blank injection. Thus, each antibody is injected twice.

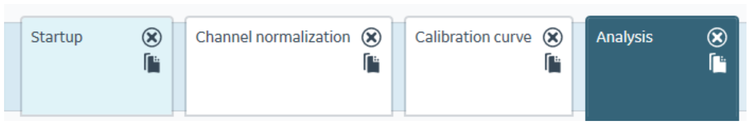


Fig 3. Assay steps required for dual parameter screening.

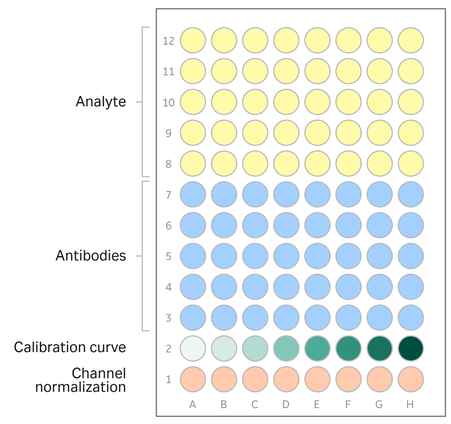


Fig 4. Example of plate layout for a dual parameter screening experiment.

Evaluating dual parameter screening

Step 1. Evaluating titer using parallel concentration analysis

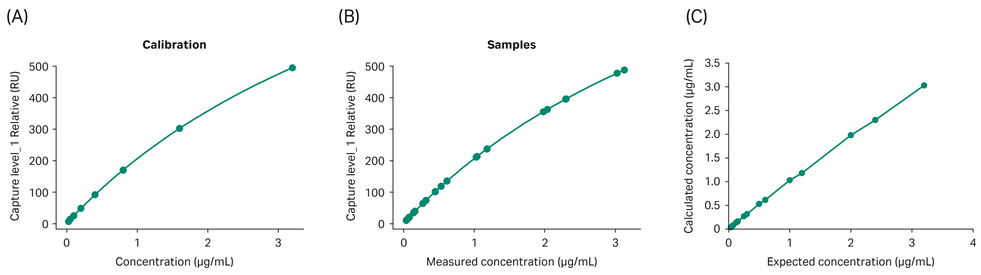


Fig 5. Assessment of antibody titer using dual parameter screening. (A) calibration curve, (B) sample response levels, (C) correlation between expected and calculated titer.

- Open analysis run data in Biacore™ Insight Evaluation Software.
- Select evaluation method for concentration analysis.
- Open concentration evaluation item and adjust Injection assignment
  - Set **Variable: *Capture***
  - Set **Data grouping: *Parallel***
- Concentration evaluation is performed automatically.

Step 2. Evaluating kinetics

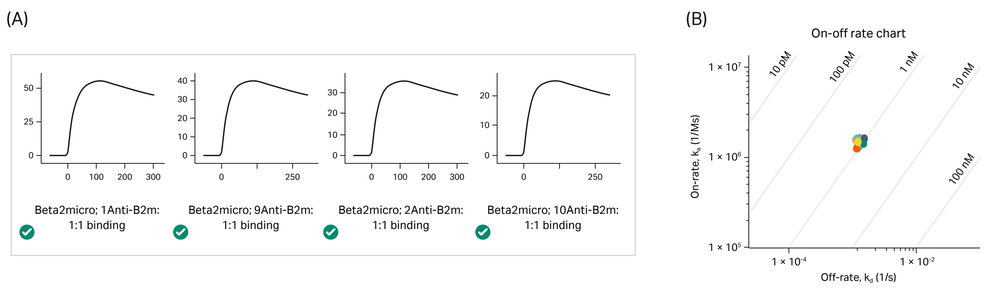
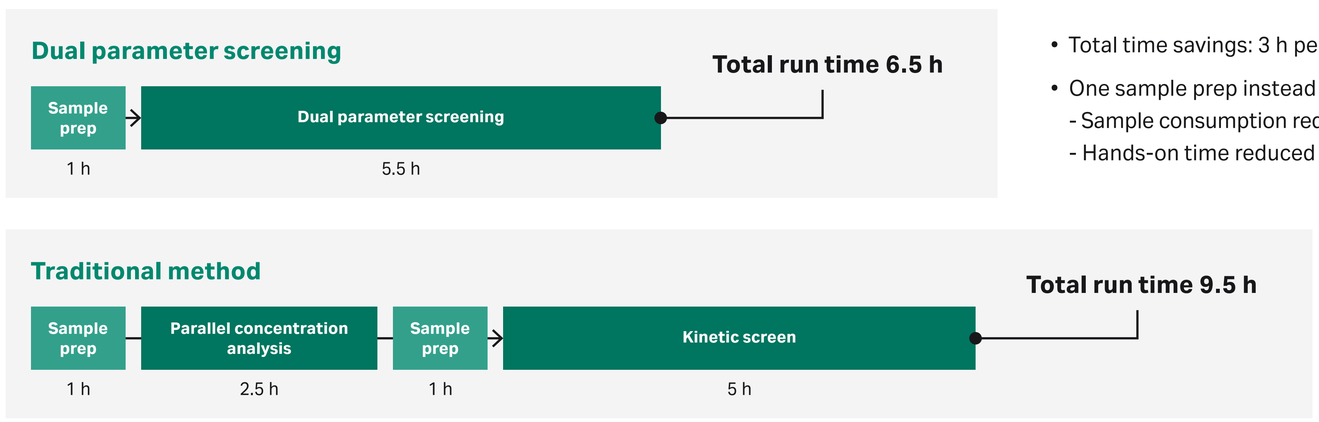


Fig 6. Assessment of kinetics using dual parameter screening. (A) Example of single concentration kinetics fits, (B) On-off rate chart showing precision of replicate measurements of an antibody sample.

- Add evaluation item for kinetics.
- Open kinetics evaluation item and adjust Injection assignment
  - Set **Variable: *Capture***
  - Set **Response: *Analyte***
  - Set **Data grouping: *Serial***
- Apply blank subtraction and fit data using 1:1 binding model.

Save time and sample using dual parameter screening

Concentration and kinetic analysis of 96 samples



- Total time savings: 3 h per 96 samples
- One sample prep instead of two
  - Sample consumption reduced by 50%
  - Hands-on time reduced by 50%

More information

Application guide, Kinetics and affinity measurements with Biacore™ systems, CY12854
Application guide, Concentration measurement with Biacore™ systems, CY12851
Biacore™ 8K+ SPR System, Cytiva, product code 29283382
Biacore™ Insight Evaluation Software, Cytiva, product code 29310603
Mouse Antibody Capture Kit, type 2, Cytiva, product code 29215281

References

1. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5368232
2. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7153844

Conclusions

Dual parameter screening delivers two antibody selection criteria in the same assay.

- Faster time to results helps you use your time more efficiently
- Saves your precious samples for complementary studies if needed
- Shorter time to better informed decisions in the selection process

cytiva.com

CY15075-16Dec21-PO

cytiva