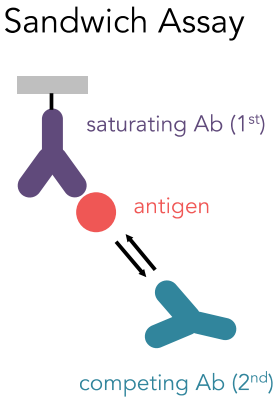
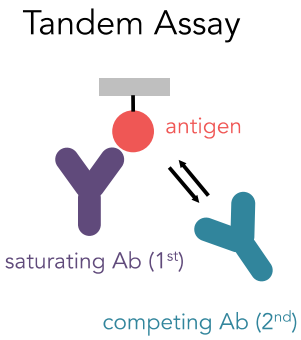
Octet RED384: CMI Getting Started Guide to Epitope Binning Biolayer Interfometry

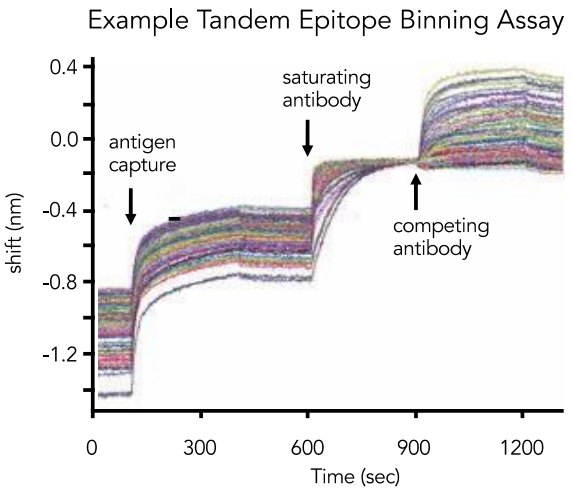
Tandem Assay Sandwich Assay Optimization Data Analysis

Introduction

An Epitope Binning assay is designed to test whether two different antibody have overlapping epitopes on an antigen of interest. Epitope Binning on the Octet RED384 (RH16) is straightforward to setup. There are two standard assays epitope binning: Tandem Assay (shown in the example below) and a Sandwich Assay.







(adapted from ForteBio)

Tandem Assay

A Tandem Epitope Binning Assay involves immobilizing the antigen (via Streptavidin or other high-affinity capture scheme) and then saturating binding with the first antibody and then tests binding in a second antibody. This works best when the off-rate for the first antibody is slow.

Step Description	[s]	Step Type
1. System Check	30	Baseline
2. Ag immobilization	300	Loading
3. Baseline	30	Baseline
4. Saturating Ab (1st Ab)	600	Association
5. Baseline	30	Baseline
6. Competing Ab (2nd Ab)	300	Association
7. (Opt.) Dissociation	600	Dissociation

Sandwich Assay

A Sandwich Epitope Binning Assays involves immobilizing the first antibody, binding of the antigen, and then binding of the second antibody. This only works for monovalent antigens (and non-aggregating samples). Depending on the capture method and the Ab type, a polyclonal blocking step may be needed after the first antibody is immobilized to ensure saturation of the sensor.

Step Description	[s]	Step Type
8. System Check	30	Baseline
9. Saturating Ab (1st Ab)	300	Loading
10. Baseline	30	Baseline
11. Blocking with polyclonal	300	Custom or Quench
12. Baseline	30	Baseline
13. Antigen	600	Association
14. Baseline	30	Baseline
15. Competing Ab (2nd Ab)	300	Association
16. (Opt.) Dissociation	600	Dissociation

Optimization and Design Considerations

Test for Self-blocking

Prior to running the binning assay, Ab1 and Ab2 concentrations should be optimized to ensure complete self-blocking. Self-blocking is when the same antibody is used as both the saturating and competing antibody. The Ab2 binding signal should be zero in this case, given that the antibody completely saturates the antigen on the biosensor as Ab1.

Concentration

In general, epitope binning experiments are performed a high concetrations relative to Kd to ensure saturation binding. Ab1 and Ab2 are typically tested at the same concentration during optimization, in the range of 100–400 nM, to ensure complete self-blocking. During the actual binning assay, however, Ab2 concentrations may be tested at one-half to one-third the concentration of Ab1 to conserve reagents. 50–200 nM of antigen is a typical concentration range for biosensor or binding.

Data Analysis

The new Octet Analysis Studio Software includes Epitope Binning Data Processing. Follow the instructions in the Chapter 7 of the Analysis Studio Software Manual (available on the desktop or within the Analysis Studio Software under Help).

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