BioTechniques In Focus

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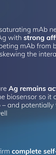
Choosing the right epitope-binning protocol

Epitope binning is a competitive immunoassay that is used to characterize the binding of monoclonal antibodies (mAbs) to a target antigen (Ag), offering information about the affinity, specificity and other biophysical characteristics of the interaction. But what protocols are available to conduct epitope binning?

In this infographic, we highlight three protocols for performing epitope binning so you can choose the right one for your research.

In-tandem assay

In this assay, the **Ag** is immobilized on the biosensor surface. Subsequently, the mAbs are introduced sequentially; the first mAb is known as the **saturating mAb** and the second is the **competing mAb**. The saturating mAb binds the Ag; upon injection of the competing mAb, the bound saturating mAb will either prevent the competing mAb from binding or not. This is then measured by the detector and reported on a sensorgram, indicating if a blocking or non-blocking response was observed, thereby informing binning.



Considerations

Ag can be **immobilized** on the biosensor directly or via tags

The saturating mAb needs to remain bound to the Ag with **strong affinity** to prevent the competing mAb from binding the free antigen and skewing the interaction data

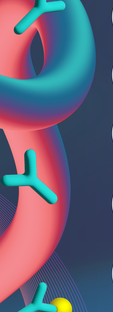
Ensure **Ag remains active** post immobilization on the biosensor so it can bind the saturating mAb – and potentially the competing mAb as well

Confirm **complete self-block**, meaning delivery of the same antibody does not displace the bound antibody

Classical sandwich assay

In this type of assay, a **mAb** is immobilized on the biosensor surface. The **target Ag** is injected and binds the immobilized mAb. A second, '**sandwiching**' **mAb** is then injected. This step determines whether the immobilized mAb, now bound to the Ag, can block the binding of the sandwiching mAb. If blocking does not occur, it indicates that the mAbs bind to different epitopes, and they are therefore categorized, or 'binned', separately.

Considerations



Ag should not rapidly dissociate from the mAb immobilized on the biosensor, so ensure immobilized mAbs bind the Ag with **strong affinity**

If using anti-human IgG Fc capture (AHC) or anti-mouse IgG Fc capture (AMC) biosensors, it is imperative to **completely block the biosensor surface** following immobilization of the first mAb as residual unblocked sites may bind directly to the sandwiching mAb, resulting in a false positive binning result

Ensure immobilization of the mAb on the biosensor doesn't cause the mAb to lose its **functionality** (i.e. it can still bind with high affinity and specificity)

Confirm there is **no cross-reactivity** between antibodies

Confirm **complete self-block**

Premix assay

In a premix assay, a **mAb** is immobilized on the biosensor surface. A **second mAb**, which has already been coupled to the **target Ag** in a premixing step, is introduced. Similar to the other assay formats, the detector measures the binding interactions and the resulting sensorgram indicates the type of blocking response. This information is once again used to inform epitope binning.

Considerations

Ag concentration must be equal to or greater than the equilibrium dissociation constant (affinity, K_D)

There should be a **molar excess** of premixed mAbs compared to the Ag

If using AHC or AMC biosensors, **ensure blocking** of unsaturated biosensor surface

Must conduct a **kinetic screen** to determine the affinity

This ensures that the Ag–mAb premixed complex completely reacts. The antigen concentration should be high enough to ensure that binding between the two molecules occurs and to enable the determination of a more accurate affinity constant (K_D), which informs premix mAb concentration.

Confirm there is **no cross-reactivity** between antibodies

Ensure **mAb remains active** post immobilization on the biosensor

Confirm **complete self-block**

Technologies for epitope binning

In addition to choosing the right kind of assay for your research, it's also important to consider what kind of technology you want to utilize. Among the many options, biolayer interferometry (BLI), surface plasmon resonance (SPR) and conventional enzyme-linked immunosorbent assays (ELISA) are the three most common technologies employed to conduct epitope binning.

	BLI	SPR	ELISA
What is it?	BLI is a fluidic-free optical biosensing technology that quantifies and analyzes interactions between an immobilized biomolecule on the sensor surface and an analyte in solution.	SPR is an optical technique used to detect and quantify molecular interactions on a surface. When an analyte solution flows over the biosensor chip, the analyte may bind to the ligand on the sensor surface, causing the refractive index to change.	ELISA is a plate-based technique that detects and quantifies biomolecules. Unlike BLI and SPR, it is not a biosensor surface-based technique and requires detection labels and several washing steps.
Pros	**Fast and real-time** **Low antigen consumption** **Label-free** **Crude lysate compatibility**	**Real-time** **Label-free** **Detection of low-affinity mAbs**	**Simple** **High throughput**
Cons	**Sensor surface artifacts can impact high-affinity measurements**	**Sensor surface artifacts can impact high-affinity measurements** **Based on microfluidics**	**Labeled reagents** **Endpoint measurement/ long run time** **Requires purified samples** **Not suitable for weak or transient interactions**
	Explore Sartorius' **BLI solutions** for epitope binning in antibody selection.	For more information about this technique, check out our **SPR In Focus**.	

When to use it

Now that we understand how these three standard protocols for epitope binning differ and the various technologies you could use, let's explore when it's appropriate to use which assay.

When deciding which format to use for binning mAbs, consider the following factors:

Sample purity

If the mAbs are in a crude form, the **classical sandwich format** might be more tolerant of impurities since the capture mAb can provide an initial purification step by binding specifically to the target Ag.

Epitope preservation

The **classical sandwich format** requires that the Ag preserves multiple epitopes in their native conformations, which might be a challenge with crude samples.

Throughput

In-tandem binning can be more suitable for high-throughput screening as it can be more easily automated and scaled.

Specificity

If there is a need to distinguish between mAbs that bind to different epitopes on the same Ag, **in-tandem binning** might provide clearer results.

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