Solid Phase Selection Guide

Selection of an optimal solid phase can increase measurement sensitivity and decrease background noise. Choosing which one will depend on the characteristics of the coating molecule that captures the constant partner in solution. Use the information below to decide what type of solid phase to try; there are a variety that can be used on the KinExA® instrument.

See *Table 1* for more information on common solid phases and their requirements.

Solid Phase Considerations

The size of the coating molecule is the first thing to consider for bead selection.

- Large molecules (>~20 kDa) and small molecules conjugated to larger proteins, such as BSA, typically adsorption coat well to hard beads (e.g. Glass and Polystyrene).
- Small, unconjugated, molecules (<~20 kDa) work best covalently coupled to soft beads (e.g. Azlactone and Sepharose).

Another consideration is if the molecule has been coupled to a plate or beads using another platform.

- Often a molecule can be coupled to the appropriate solid phase using the same immobilization chemistry as an ELISA plate or SPR surface.
- If pre-coated beads for an affinity column are at hand, they can be used for a KinExA experiment. Bead requirements can be read in the self titled section on Page 2.

If the coating material is expensive or limiting, try reversing the assay first. If it cannot be reversed, search for a biotinylated version of the molecule.

- Biotinylated molecules can be coated onto “hard” beads that have first been coated with Streptavidin.
- For more information on this coating procedure see How to Guide 208 *Biotinylated Coating* (**HG208**).

Solid phase	Type	Particle Size	Coating Method	Molecule Quantity	Molecule Requirements	Instrument Sample Limits	Coating Procedure
Polystyrene	Hard	98 µm	Adsorption	30 µg	>20 kDa	≤3 mL/min sample flow rate; ≤5 mL sample volume	HG207
Glass	Hard	53-63 µm	Adsorption	30 µg	>20 kDa	≤3 mL/min sample flow rate; ≤5 mL sample volume	HG207
Streptavidin Coated Polystyrene	Hard	98 µm	Adsorption	15-30 µg	Biotinylation	≤3 mL/min sample flow rate; ≤5 mL sample volume	HG208
Azlactone	Soft	50-80 µm	Covalent	10-20 µg	Primary Amine ($-NH_2$)	≤1 mL/min sample flow rate; ≤4 mL sample volume	HG209
Sepharose	Soft	45-165 µm	Covalent	10-20 µg	Primary Amine ($-NH_2$)	≤1 mL/min sample flow rate; ≤4 mL sample volume	HG210
CNBr-activated Sepharose	Soft	45-165 µm	Covalent	10-20 µg	Primary Amine ($-NH_2$)	≤1 mL/min sample flow rate; ≤4 mL sample volume	Manufacturers Instructions
CM Sepharose	Soft	45-165 µm	Covalent	10-20 µg	Carboxymethyl ($-CH_2-COOH$)	≤1 mL/min sample flow rate; ≤4 mL sample volume	Manufacturers Instructions
SulfoLink	Soft	45-165 µm	Covalent	10-20 µg	Sulfhydryl (-SH)	≤1 mL/min sample flow rate; ≤4 mL sample volume	Manufacturers Instructions

Table 1. *Commonly used solid phases and their requirements for KinExA measurements*

In some cases, adsorption coating and/or covalent coupling through primary amines can mask the binding site of interest. In that case, check if there are different functional groups available on the molecule.

- If there are free functional groups available for coupling, that won't interfere with the binding site/epitope, they can be used to covalently couple the molecule to soft beads or biotin.
- There are various types of soft beads that can bind different functional groups. For example, CM Sepharose couples via carboxymethyl group.
- There are a wide range of biotinylation reagents available to target specific functional groups. For more information on biotinylation reagents go to LifeTechnologies.com and search "Thermo Scientific Avidin-Biotin Technical Handbook".

Bead Requirements

Beads other than the ones listed in Table 1 can be used. Consider the following:

- The beads must be >20 micron or they will not be retained in the flow cell.
- Avoid fluorescent beads as they will affect the measurement.
- Avoid magnetic beads as they absorb light and affect the measurement.