Solid Phase Selection Guide

Selecting the optimal solid phase can increase measurement sensitivity and decrease background noise. The type of solid phase to select will depend on the characteristics of the coating material used to capture the constant binding partner in solution. Use the information below to decide what type of solid phase to try. There are a variety of different solid phases 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 molecule to be coated 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. PMMA and Polystyrene).
- Small, unconjugated molecules (<~20 kDa) work best covalently coupled to soft beads (e.g. Azlactone and Sepharose).

Another good starting point is to identify if the molecule has been coupled to a plate or beads using another platform.

- Many times you can couple molecules to the appropriate solid phase using the same immobilization chemistry as an ELISA plate or SPR surface.
- If you already have coated beads for an affinity column then you can use those beads for your KinExA experiment. Requirements for these beads and others can be seen in the section titled "Bead Requirements".

If the coating material is expensive or limiting you should try reversing the assay first. If it cannot be reversed, you should see if there is a biotinylated version of the molecule available.

- Biotinylated molecules can be coated onto PMMA beads that have first been coated with streptavidin. The amount of biotinylated material needed for coating is lower (10-30 µg) than for standard PMMA (30 µg).
- For more information on coating these types of beads see How to Guide 208 *Biotinylated PMMA Coating* (**HG208**).

Solid Phase Name	Type	Particle Size Distribution	Quantity of Coating Material	Bound By	Coating Material Requirements	Sample Condition Limits	Coating Procedures
PMMA/ Polystyrene	Hard	90 to 106 µm	30 µg	Adsorption	>~20 kDa	≤3 mL/min flow rate	HG207
Streptavidin coated PMMA	Hard	90 to 106 µm	10-30 µg	Adsorption/ Avidin-biotin interaction	Biotinylated molecule	≤3 mL/min flow rate	HG208
Azlactone	Soft	50 to 80 µm	10-20 µg	Covalent	Primary amine ($-NH_2$)	≤1 mL/min flow rate; ≤5 mL volume	HG209
Sepharose	Soft	45 to 165 µm	10-20 µg	Covalent	Primary amine ($-NH_2$)	≤1 mL/min flow rate; ≤5 mL volume	HG210
CM Sepharose	Soft	45 to 165 µm	10-20 µg	Covalent	Carboxymethyl ($-CH_2-COOH$)	≤1 mL/min flow rate; ≤5 mL volume	Manufacturer's Instructions
CNBr-activated Sepharose	Soft	45 to 165 µm	10-20 µg	Covalent	Primary amine ($-NH_2$)	≤1 mL/min flow rate; ≤5 mL volume	Manufacturer's Instructions
SulfoLink	Soft	45 to 165 µm	10-20 µg	Covalent	Sulfhydryl (-SH)	≤1 mL/min flow rate; ≤5 mL volume	Manufacturer's Instructions

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

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

- If there are any free functional groups available for coupling, that won't interfere with the binding site/epitope, they can be used to covalently bind the molecule to soft beads or biotin.
- There are various types of soft beads that can bind many 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

There are other bead types besides the ones listed in ***Table 1*** that will work with the KinExA, however there are a few requirements to consider.

- The beads must be larger than 20 micron in order to be retained in the flow cell.
- If the beads fluoresce, they can affect the measurement.
- If the beads are magnetic they can absorb light, making them appear black, which can affect the measurement.