

Biotinylated Coating

In some cases it is advantageous to use a biotinylated form of the Titrant for the solid phase. The linker arm to the biotin can expand the space between the molecule and the solid phase. The additional space allows the molecule more freedom to interact with the molecules in solution (Figure 1). A biotinylated form of the molecule may be readily available, or it can be biotinylated with simple to use kits. Different kits, targeting different reactive groups, are available from Life Technologies' website (search "Avidin-Biotin handbook"). In these cases, using a biotinylated form of the molecule is sufficient and sometimes preferred as the capture reagent. Hard Beads¹ can be coated with biotinylated molecules using the following protocol. Sapidyme offers precoated Glass Streptavidin Beads for purchase (446101). To skip steps 1 and 2, see *Streptavidin Coated Glass Beads* (HG276).

Step 1. BSA-Biotin Coating

- To [1] vial of hard beads, add **1 mL** of BSA-Biotin coating solution. This solution consists of **20 µg/mL** of BSA-Biotin² Conjugate in 1x PBS, pH 7.4, 0.02% NaN₃. Make sure the beads are fully suspended in solution.
- Rock/tumble bead vials at room temperature for **1 hour** or overnight at **4°C**. Sapidyme recommends using a Tube Rotator (840751).
- Allow the beads to settle or pulse centrifuge at a low speed to pellet the beads. Discard the supernatant without disturbing the settled beads.

Step 2. Avidin Coating

- Add **1 mL** of Avidin coating solution. This solution consists of **100 µg/mL** Neutravidin or Streptavidin³ coating solution in 1x PBS, pH 7.4, 0.02% NaN₃ with **10 mg/mL** BSA⁴. Make sure the beads are suspended in the coating solution.
- Rock/tumble bead vials at room temperature for **1 hour** or overnight at **4°C**.

- Allow the beads to settle or pulse centrifuge at a low speed to pellet the beads. Discard the supernatant without disturbing the settled beads.

Step 3. Biotinylated Molecule Coating

- Prepare **1 mL** of **15-30 µg/mL** biotinylated molecule in 1x PBS, pH 7.4, 0.02% NaN₃.
Note: Quantity of coating material is suggested as a good compromise between signal and material. Coating with more material will not significantly increase the signal, however, coating with less will decrease the signal and the linear range of usable signals. For more information on linear range, see *Linear Range* (TN224).
- Aliquot the biotinylated molecule coating solution into the vial containing the Avidin-Coated Beads. Ensure that the beads are suspended in the coating solution.
- Rotate beads for **30 minutes** at room temperature or overnight at **4°C**.

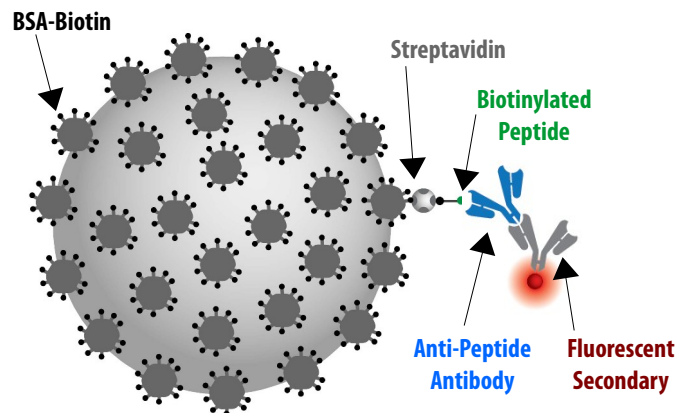


Figure 1. Example of an assay format using biotinylated-coated beads.

- Glass Beads Pack of 10: Sapidyme (440425).
Polystyrene Beads Pack of 10: Sapidyme (442178).
Note: Sapidyme Does not sell PMMA Beads.
- BSA-Biotin: Millipore Sigma (A8549-10MG).
- Streptavidin: Jackson ImmunoResearch (016-000-114).
- Bovine Serum Albumin (BSA): Sapidyme (2A3017).