

Biotinylated PMMA 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. 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. PMMA beads can be coated with biotinylated molecules using the following protocol.

Step 1. BSA-Biotin Coating

- a) Prepare 1 mL of 20 µg/mL BSA-biotin conjugate in 1x PBS, pH 7.4 (Part #: 270105).
- b) Aliquot the BSA-biotin coating solution into [1] vial of PMMA beads (Part #: 440176). Ensure that the beads are suspended in the coating solution.
- c) Rotate beads for 2 hours at room temperature or overnight at 4°C.
- d) Allow the beads to settle or pulse centrifuge at a low speed to pellet the beads. Remove the supernatant without disturbing the settled beads.
- e) Rinse beads five times with 1 mL aliquots of 1x PBS, pH 7.4, spinning and discarding the supernatant each time.

Step 2. Avidin Coating

- a) Prepare 1 mL of 100 µg/mL neutravidin or streptavidin coating solution in 1x PBS, pH 7.4 with 10 mg/mL BSA.
- b) Aliquot the neutravidin or streptavidin coating solution into the vial containing BSA-biotin PMMA particles. Ensure that the beads are re-suspended in the coating solution.
- c) Rotate beads for 1 hour at room temperature or overnight at 4°C.
- d) Allow the beads to settle or pulse centrifuge at a low speed to pellet the beads. Remove the supernatant without disturbing the settled beads.
- e) Rinse beads five times with 1 mL aliquots of 1x PBS, pH 7.4, spinning and discarding the supernatant each time.

Step 3. Biotinylated Molecule Coating

- a) Prepare 1 mL of 15-30 µg/mL* biotinylated molecule in 1x PBS, pH 7.4.
- b) Aliquot the biotinylated molecule coating solution into the vial containing the avidin-coated PMMA particles. Ensure that the beads are re-suspended in the coating solution.
- c) Rotate beads for 1 hour at room temperature or overnight at 4°C.

**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 Technical Note 224 Linear Range (TN224).*