

SAMPLE PROTOCOL FOR BINDING BIOTIN-CONJUGATED MOLECULES TO LUMAVIDIN MICROSPHERES

Microspheres should be protected from prolonged exposure to light throughout this procedure.

1. Resuspend the stock LumAvidin microsphere suspension according to the instructions described in the Product Information Sheet provided with your microspheres.
2. Transfer 1.0×10^5 of the stock microspheres to a **USA Scientific** microcentrifuge tube.
3. Pellet the stock microspheres by microcentrifugation at $\geq 8000 \times g$ for 1-2 minutes.
4. Remove the supernatant and resuspend the pelleted microspheres in 250 μL of PBS-1% BSA by vortex and sonication for approximately 20 seconds.
5. Dilute the biotin-conjugated molecule in PBS-1% BSA to a concentration of 4 to 4000 nM. (Note: We recommend titration in the 4 to 4000 nM range to determine the optimal amount of biotin-conjugated molecule per specific binding reaction.)
6. Add 250 μL of the biotin-conjugated molecule solution to the microsphere suspension and mix immediately by vortex.
7. Incubate for 30 minutes with mixing (by rotation) at room temperature.
8. Pellet the bound microspheres by microcentrifugation at $\geq 8000 \times g$ for 1-2 minutes.
9. Remove the supernatant and resuspend the pelleted microspheres in 500 μL of PBS-TBN by vortex. See **Technical Note 1**.
10. Pellet the bound microspheres by microcentrifugation at $\geq 8000 \times g$ for 1-2 minutes.
11. Repeat steps 9. and 10. This is a total of two washes with PBS-TBN.
12. Remove the supernatant and resuspend the microspheres in 250-1000 μL PBS-TBN by vortex and sonication for approximately 20 seconds.
13. Count the microsphere suspension by hemacytometer.

Calculation: Total microspheres = count (1 corner of 4 x 4 section) x (1×10^4) x (dilution factor) x (resuspension volume in mL)

14. Store the bound LumAvidin microspheres refrigerated at 2-8°C in the dark.

Technical Note 1: Either PBS-TBN (PBS, 0.1% BSA, 0.02% Tween-20, 0.05% Azide, pH 7.4) or PBS-BN (PBS, 1% BSA, 0.05% Azide, pH 7.4) may be used as Wash and Blocking/Storage Buffer.