Protocol: Binding biotinylated nucleic acids, antibodies, or proteins to Streptavidin Magnetic Beads for Pull-down Experiments (NEB #S1420)

Overview

Materials:	NEB Product Number
Streptavidin Magnetic Beads	S1420S
Magnetic rack sized appropriately for your experiment:	
96-well Microtiter Plate Magnetic Separation Rack	S1511S
6-tube Magnetic Separation Rack for 1.5 mL microfuge tubes	S1506S
12-tube Magnetic Separation Rack for 1.5 mL microfuge tubes	S1509S
50 mL Magnetic Separation Rack for 50 mL conical tubes	S1507S
Biotinylated bait (nucleic acid, antibody or protein)	
Binding/Wash buffer for nucleic acids: 10 mM Tris-HCl pH 7.5, 1 M NaCl, 1 mM EDTA	
Binding/Wash buffer for antibodies or proteins: 1X PBS pH 7.4 (+ 0.01% w/v Recombinant Albumin and/or 0.05% Tween-20 to reduce non-specific binding)	
Optional: NanoDrop/spectrophotometer	

Protocol:

1. Vortex/mix the tube containing the Streptavidin Magnetic Beads well to fully resuspend them.

2. Transfer the required volume of beads for your experiment to a microfuge tube. Streptavidin Magnetic Beads bind 500 pmol of 25 bp single stranded DNA (ssDNA) or 30 µg of biotinylated antibody or protein per milligram of beads; the beads are supplied at 4 mg/mL.

   Note: Optimal loading density of biotinylated bait on beads needs to be determined empirically. We recommend these values as a starting point

3. Place the tube in the magnetic rack and allow the beads to pellet; remove the storage buffer.

4. Remove the tube from the magnetic rack. Equilibrate the beads with binding/wash buffer by mixing the beads thoroughly in a volume of binding buffer equal to or greater than the volume of beads transferred in step 2.

5. Place the tube in the magnetic rack and allow the beads to pellet. Remove the binding wash/buffer.

6. Repeat steps 4 and 5 twice more for a total of 3 equilibration washes.

7. Resuspend the biotinylated bait (nucleic acid/antibody/protein) in appropriate binding buffer (adjusting the NaCl concentration of this solution to be between 0.5 and 1 M after resuspension for nucleic acid binding)

   Optional: Measure the A_{260} (nucleic acid) or A_{280} (antibody/protein) of the resuspended biotinylated bait to determine amount of bait bound

8. Add resuspended biotinylated bait to pelleted and equilibrated magnetic beads. Mix thoroughly by pipet mixing or gentle vortexing.

9. Incubate with mixing (rotisserie-style end-over-end mixing or ThermoMixer/equivalent) for > 30 minutes at room temperature or 4C, depending on the stability of your biotinylated bait.

10. Place the tube in the magnetic rack and allow the beads to pellet.

    Optional: Measure the A_{260} or A_{280} of the solution to quantify how much biotinylated bait remains. The difference between the first measurement and this measurement is a good approximation of the amount of biotinylated bait bound to the Streptavidin Magnetic Beads

11. Wash the beads with the appropriate binding/washing buffer (or a wash buffer of higher stringency) as in steps 4-6.

12. Resuspend the beads in a buffer compatible with your specific pull-down experiment; proceed directly to the pull-down experiment.