Product Name	Cat. No.	Pack Size
Streptavidin Magnetic Beads	**# BB-MSA01A**	**0.25 ml Packed Bead Volume**
	# BB-MSA01B	**0.5 ml Packed Bead Volume**
	# BB-MSA01C	**1 ml Packed Bead Volume**

Introduction:

Our Streptavidin-bound Magnetic Agarose Beads are useful for immunoprecipitation/pull down assay of biotin tagged proteins or nucleic acids.

Product Description:

Approximately 1.5 mg/ml streptavidin is immobilized on non-cross-linked beaded magnetic agarose where iron is encapsulated by agarose. The beads have excellent magnetic properties allowing rapid and efficient capture and low non-specific binding using a Magnetic Bead Stand*. These beads are stable at pH 2-11. The product is supplied as 50% suspension of streptavidin magnetic agarose in 20% ethanol aqueous suspension when packed.

*BBL also sells Magnetic Bead Stand

TECHNICAL SPECIFICATIONS

BEAD GEOMETRY & SIZE	: Spherical, ~ 5 - 10 µm diameter (Encapsulated with Iron)
CROSSLINKED	: NO
ACTIVATING GROUP	: Carbonyl
MATRIX STABILITY	: Stable in all commonly used reagents
STORAGE SOLUTION	: 20% ethanol aqueous
STORAGE TEMPERATURE	: 4°C to 8°C. **DO NOT FREEZE**.
STABILITY	: Stable for 1 year under proper storage condition

Please Note:

- To elute biotinylated molecules from the streptavidin resins, use 8M guanidine• HCl, pH 1.5 or extract the pulled magnetic beads in SDS-PAGE sample buffer at 70^{O}C for 5 minutes.
- Guanidine• HCl may irreversibly damage the protein of interest. Furthermore, this harsh elution condition may result in leaching of streptavidin subunits and a considerable reduction in resin binding capacity from the loss of these subunits

General Description of Immunoprecipitation or Pull-down assay

- Prepare cell lysate at roughly 1 - 4 µg/µl of total cellular protein. Add 0.5 mg -1.0 mg of total protein to a microcentrifuge tube.

  To Make Cytoplasmic Extracts:

  1. Remove media from the plate and wash cells with PBS.
  2. Add 1 ml PBS + 1 mM EDTA
  3. Scrape cells and transfer to 1.5 µL centrifuge tube; spin down in microfuge for 30 sec. Remove supernatant.

  Make cytoplasmic extract by adding 400 µL cytoplasmic extraction (CE) buffer and resuspend. (CE=10mM HEPES-KOH pH7.9, 250mM NaCl, 0.5% NP-40, 0.2% Tween 20, 1mM EDTA with freshly added Protease Inhibitor cocktail, 2 mM DTT).
  [If working with phospho protein addition of 1 mM PMSF, 10 mM NaF, 20 mM β-glycerophosphate, 0.1 mM Na_3VO_4 (phosphatase inhibitor) is needed].

  4. Keep in ice for 2 min.
  5. Vortex 1 min at a moderate setting.
  6. Clarify the lysate by centrifugation at 14,000 x g for 2 minutes at 4°C and transfer the supernatant to a fresh tube.
  7. Measure protein concentrations of the lysate using Bradford assay.

  Pre-clearing the lysate: Add ~20 µL bead slurry (~10 uL packed beads) in CE buffer per 100-500 µL cytoplasmic extract. Gently vortex and incubate at 4°C for 1 hour on rotor. Place the tube on a magnetic stand for 30 seconds to pull down the beads to the side of the tube. Transfer pre-cleared lysate to a fresh tube and discard the magnetic bead pellet.
- Add appropriate amounts of biotinylated molecule (peptide, protein, antibody, DNA and RNA) to the pre-cleared lysate fraction.
- Gently rock the reaction mixture at 4°C overnight (time of incubation can be reduced).
- Capture the immune complex by adding **20 µl of washed Streptavidin-Magnetic-Agarose** bead slurry (10 µl packed bead).
- Gently rock the reaction mixture at 4°C for 1-2 hours.
- Place tubes containing magnetic agarose beads in the Magnetic Bead Stand. Wait 1 to 2 minutes for the magnetic beads to be settled and the solution becomes clear. Carefully remove the supernatant, **while the tube is resting in the stand.**
- Remove the tubes from the stand and wash the beads by re-suspending with either ice-cold cell lysis buffer or PBS by gently vortexing or rocking the tube. Carefully drain the supernatant by placing the tubes in the stand as described in above. Repeat the washing steps for 3 times.
- Treat the pulled magnetic beads in SDS-PAGE sample buffer (DTT concentration ~150 mM), at **70^{O}C for 5 minutes** and collect the extracted samples while keeping the tubes in the magnetic bead stand followed by SDS-PAGE.
- Bound molecules can be visualized by Western blot or Coomassie or Silver staining methods. (The method of choice should be determined by the investigator)
- Bound unknown proteins can also be analyzed by mass-spectrometry where specific bands(s) can be isolated from the gel for mass-spec or all bound samples in the bead can be subjected to mass-spec.