

Product Name	Cat. No.	Pack Size
<b>Streptavidin Agarose</b>	# BB-SAG001A	<b>0.5 ml Packed Bead Volume</b>
	# BB-SAG001B	<b>1 ml Packed Bead Volume</b>
	# BB-SAG001C	<b>2 ml Packed Bead Volume</b>
	# BB-SAG001D	<b>5 ml Packed Bead Volume</b>

**Introduction:** Our Streptavidin bound Agarose beads are useful for assay development using affinity chromatography purifications of biotin tagged proteins or nucleic acid and immunoprecipitation.

Recombinant streptavidin purified from *E.coli* is covalently bound to 6% cross-linked agarose bead. The Streptavidin-linked resin which is stable at pH 2-11 can be used to separate biotinylated products from non-biotinylated products. Biotinylated molecules can be RNA, DNA or protein which binds to the Streptavidin agarose bead with extremely high affinity. This high affinity interaction is useful to monitor protein-RNA, protein-DNA and protein-protein interaction.

**Product Description:** Approximately 1.5 mg/ml streptavidin has been immobilized on 6% crosslinked beaded agarose. This product is supplied as 50% suspension of Streptavidin Resin in aqueous ethanol when packed.

**TECHNICAL SPECIFICATIONS**

BEAD GEOMETRY & SIZE	: Spherical, ~ 50 - 150 µm diameter
CROSSLINKED	: Yes
BEAD CROSSLINKING %	: 6%
ACTIVATING GROUP	: Carbonyl
MATRIX STABILITY	: Stable in all commonly used reagents
STORAGE SOLUTION	: 20% aqueous ethanol
STORAGE TEMPERATURE	: 4°C to 8°C. <b>DO NOT FREEZE.</b>
STABILITY	: Stable for 1 year under proper storage condition

**General Description of Immunoprecipitation or Pull-down assay**

- ❖ Prepare a cell lysate at roughly 1 - 4 µg/µl of cellular protein, and add 500 µg-1mg of protein to a microcentrifuge tube.
- ❖ Clarify the lysate by centrifugation at 14,000 X g for 2 minutes at 4°C.
- ❖ Transfer the supernatant fraction to another microcentrifuge tube.
- ❖ Add appropriate amounts of biotinylated molecule (peptide, protein, antibody, DNA and RNA) to the supernatant fraction.
- ❖ Gently rock the reaction mixture at 4°C overnight (time of incubation can be reduced).
- ❖ Capture the immune complex by adding **20-100µl of washed Streptavidin-Agarose** bead slurry (10-50 µl packed bead).
- ❖ Gently rock the reaction mixture at 4°C for 1-2 hours.
- ❖ Collect the agarose beads by pulsing (15 seconds in the microcentrifuge at 14,000 X g), and drain off the supernatant. Wash the beads 3 times with either ice-cold cell lysis buffer or PBS.



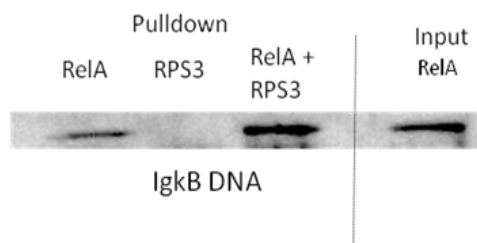
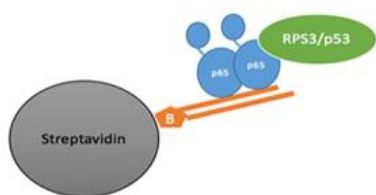
## Protocol: Antibody Purification using Protein G<sup>PLUS</sup> Agarose Beads

- ❖ Boil the agarose bead in SDS-PAGE sample loading buffer for 5 minutes 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.

**One Specific Example:** NF- $\kappa$ B RelA subunit is a DNA binding transcription factor. RPS3 is a cofactor of RelA which induced DNA binding of RelA. RPS3 does not bind DNA. Pull down experiments were performed to show that RelA binds Ig- $\kappa$ B DNA (specific RelA binding sequence present in the promoter of immunoglobulin kappa light chain gene).

## Pulldown Experiments

- Pulldown experiments using streptavidin beads and biotinylated DNA *in vitro* show that NF- $\kappa$ B RelA homodimer (baculovirus-derived full length protein) binds Ig- $\kappa$ B DNA and that RPS3 enhances RelA's binding to  $\kappa$ B DNA.



10  $\mu$ l streptavidin bead slurry/reaction,  
0.5  $\mu$ l of 50  $\mu$ M biotin DNA/reaction  
100  $\mu$ l binding reaction

### Binding Buffer:

150 mM NaCl, 25 mM Tris 7.5, 10 % glycerol, 0.1 % NP40, 1 mM DTT, 0.2 mM EDTA

### Wash Buffer:

Binding Buffer but with 100 mM NaCl

#### 1. Pre-binding/blocking of beads:

10  $\mu$ l of streptavidin bead slurry washed with binding buffer and brought to 1 ml in blocking buffer (binding buffer + 5% BSA)

3  $\mu$ l of 50  $\mu$ M annealed biotinylated DNA added and rotated for 1 hour at 4 C

Beads washed 4 times with binding buffer to remove excess BSA/unbound biotinylated DNA. Beads resuspended in a final volume of 20  $\mu$ l in binding buffer.

#### 2. Binding of protein to beads:

Add 180  $\mu$ l of binding buffer to 20  $\mu$ l of the previously prepared bead slurry (200  $\mu$ l final volume/used nuclear extract fractions instead of binding buffer)

Add 2 ng/  $\mu$ l sonicated salmon sperm DNA and 100 nM of annealed mutated nonspecific DNA

2  $\mu$ g of RelA or RPS3 or RelA+RPS3 added per reaction

Rotate for 1 hour at 4C

Pellet beads and wash twice with 400  $\mu$ l wash buffer (binding buffer with 100 mM NaCl)

After final wash, add 4 X SDS dye to 1 X final, and load onto SDS-PAGE for protein separation.

Bound protein (RelA) was visualized by WB using anti-RelA antibody.