# Streptavidin Beads C3

## Introduction

APExBIO Streptavidin Beads C3 are ideal for nucleic acid applications (specifically for samples with a high chaotropic salt concentration), immunoassays involving small biotinylated antigens and applications that are not compatible with bovine serum albumin (BSA) (these beads are not blocked with BSA).

Add the beads to a sample containing biotinylated molecules, e.g. peptides or oligonucleotides. During a short incubation, the biotinylated molecule binds to the beads. The molecule-bead complex can be separated by using a magnet. Capture, washing, and detection can be optimized for manual or automated use. With indirect target capture, mix the biotinylated molecule with the sample to capture the molecule-target complex before adding the beads.

## Components and Storage

| Name | V (mL) | Concentration | Supplied in | Bead diameter (μm) | Storage | Recommended protein binding amount |
|---|---|---|---|---|---|---|
| Streptavidin Beads C3 | 1 or 5*1 mL | 10 mg/mL | PBS, pH 7.4 / 0.14 M NaCl / 0.02% $NaN_3$ | 3 | Store at 2-8˚C | 10 μg IgG/mg |

*Note: APExBIO M-270 Streptavidin supplied in 0.0065 M phosphate buffer pH 7.4, with 0.14 M NaCl and 0.02% sodium azide as a preservative.

CAUTION: Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

## Characteristics and properties

- Hydrophilic bead surface
- Based on carboxylic acid beads
- No blocking proteins used
- Isoelectric point: pH 4.5
- High charge (–50 mV, at pH 7)
- Iron content (ferrites): 14% (20%)
- Low aggregation of beads in high-salt solutions

## Protocol

**Washing buffers preparation**

1. Nucleic acid applications: 2× B&W Buffer (see Table 1). Dilute to 1× B&W Buffer with distilled water.
2. Antibody/protein applications: PBS, pH 7.4.

Table 1. Washing buffers preparation

| For coupling of nucleic acids | For RNA applications |
|---|---|
| Binding and washing (B&W) Buffer (2×):<br>10 mM Tris-HCl (pH 7.5)<br>1 mM EDTA<br>2 M NaCl | Solution A:<br>DEPC-treated 0.1 M NaOH<br>DEPC-treated 0.05 M NaCl<br><br>Solution B:<br>DEPC-treated 0.1 M NaCl |
| *Note: The salt concentration and pH (typically 5-9) of the chosen binding/washing buffers can be varied depending on the type of molecule to be immobilized. Beads with immobilized molecules are stable in common buffers. | |

### Wash APExBIO magnetic beads

1. Vortex for more than 30 s, or tilt and rotate for 5 min to resuspend the beads in the vial.
2. Calculate the amount of beads required based on their binding capacity, and transfer the beads to a new tube.
3. Add an equal volume of B&W Buffer, or at least 1 mL B&W Buffer to resuspend beads.
4. Place the tube on a magnet for 1 min and then remove the supernatant carefully.
5. Remove the tube from the magnet. Add the same volume of B&W Buffer as the initial volume of beads taken from the vial (step 2) to resuspend beads.
6. Repeat steps 4-5 twice, for a total of 3 washes.

### APExBIO magnetic beads for RNA manipulation

APExBIO Streptavidin are ***not*** supplied in RNase-free solutions. When using for RNA applications, please perform the following steps after washing:

1. Wash the beads twice in Solution A for 2 min. Use the same volume (or greater) of Solution A as the initial volume of beads taken from the vial.
2. Wash the beads once in Solution B. Use the same volume as with Solution A.
3. Resuspend the beads in Solution B.

The beads are now ready to be coated with the biotinylated molecule of your choice.

### Immobilization protocol

Wash the APExBIO magnetic beads according to “Wash APExBIO magnetic beads” section before use.

1. Add the biotinylated molecule to the washed beads.
2. Incubate for 15-30 min at room temperature with gentle rotation of the tube.
3. Place the tube in a magnet for 2-3 min and discard the supernatant.
4. Wash the coated beads 3-4 times in B&W Buffer.
5. Resuspend to desired concentration in a suitable buffer for your downstream use.

Here are some examples of immobilization protocols for specific applications.

### Immobilize nucleic acids

1. Resuspend beads in 2× B&W Buffer to a final concentration of 5 μg/μL.

2. To immobilize, add an equal volume of the biotinylated DNA/RNA in distilled water to dilute the NaCl concentration in the 2× B&W Buffer from 2 M to 1 M for optimal binding.

3. Incubate for 15 min at room temperature using gentle rotation. Incubation time depends on the nucleic acid length: short oligonucleotides (<30 bases) require max. 10 min. DNA fragments up to 1 kb require 15 min.

4. Separate the biotinylated DNA/RNA coated beads with a magnet for 2-3 min.

5. Wash 2-3 times with 1× B&W Buffer.

6. Resuspend to the desired concentration. Binding is now complete. Resuspend the beads with the immobilized DNA/RNA fragment in a buffer with low salt concentration, suitable for downstream applications.

**Immobilize antibodies/proteins**

1. Incubate the beads and biotinylated antibodies in PBS for 30 min at room temperature using gentle rotation.

2. Separate the antibody-coated beads with a magnet for 2-3 min.

3. Wash the coated beads 4-5 times in PBS containing 0.1% BSA.

4. Resuspend to the desired concentration for your application.

## Note

1. Keep the tube on the magnet for 2 min to ensure that all the beads are collected on the tube wall.

2. For diluted samples, increase the incubation time or divide the sample into several smaller aliquots.

3. Avoid air bubbles during pipetting.

4. Free biotin in the sample reduces the binding capacity of the beads. A disposable separation column or a spin column can be used to remove unincorporated biotin.

5. For some applications, it can be an advantage to add a detergent such as 0.01-0.1% Tween-20 to the washing/binding buffers to reduce non-specific binding.

6. Run the PCR with limiting concentrations of biotinylated primer, or remove free biotinylated primer by ultrafiltration, microdialysis, or other clean-up protocols.

7. Use a mixer to tilt/rotate the tubes so magnetic beads do not settle at the bottom of the tube.

APExBIO Technology
www.apexbt.com
7505 Fannin street, Suite 410, Houston, TX 77054.
Tel: +1-832-696-8203 | Fax: +1-832-641-3177 | Email: info@apexbt.com