

# Streptavidin and Biotin Conjugated Microspheres

## DESCRIPTION

Polysciences, Inc. offers streptavidin and biotin covalently coupled to undyed and fluorescently dyed microspheres. Streptavidin coated microspheres can be used to capture biotinylated compounds, whereas the biotin microspheres will bind streptavidin labeled components, complexes, or cells. Both these types of microspheres are offered as aqueous suspensions containing microspheres at a concentration of ~1.25%. They are packaged in a 0.02M sodium phosphate buffer (pH 7.4), containing 8 mg/ml NaCl,

10 mg/ml Bovine Serum Albumin (BSA), 0.1% sodium azide and 5% glycerol. The fluorescent yellow-green (YG) microspheres have an excitation max of 441nm and an emission max of 486nm, similar to FITC. Each lot of the microspheres is quality control tested for mean particle diameter, percent solids, and binding capacity.

## CHARACTERISTICS

Particle Concentration: 1.25%

## MATERIAL

### Material Supplied

- Streptavidin or biotin conjugated microspheres

### Material Required

- 100ml PBS/BSA binding buffer
- 100ml graduated cylinder
- Microcentrifuge tubes
- Microcentrifuge

## PROCEDURE

Researchers are advised to optimize the use of particles in any application.

### Preparation of Solution

1. Prepare 0.1 M phosphate buffer (pH 7.4) by adding 0.1 M  $\text{NaH}_2\text{PO}_4$  to 0.1 M  $\text{Na}_2\text{HPO}_4$  until pH reaches 7.4.
2. Place 20ml of the 0.1 M phosphate buffer in a 100ml graduated cylinder.
3. Add 0.88g NaCl and 1g BSA and make up the volume to 100ml using phosphate buffer.
4. Check pH of final solution. If necessary, adjust pH to 7.4 by adding dilute HCl or NaOH.

### Streptavidin Conjugated Microspheres

The widespread availability of biotinylated antibodies and their use with streptavidin conjugated microspheres facilitate procedures such as cell sorting and immunoprecipitation. Streptavidin conjugated microspheres

can also be used with individual biotinylated antibodies or in procedures that utilize a mixture of biotinylated antibodies. Polysciences' streptavidin conjugated microspheres can be used as a solid mobile phase. The streptavidin-biotin bond strength approaches that of a covalent bond, with an association constant of  $K_a = 10^{15} \text{ Mol}^{-1}$  in an aqueous solution. This makes the bond essentially irreversible in addition to being an interaction of high affinity and specificity. The streptavidin-biotin complex is stable over a wide range of pH and temperatures. Both plain and YG streptavidin conjugated microspheres are available in 1 $\mu\text{m}$ , 2 $\mu\text{m}$  and 6 $\mu\text{m}$  diameters. The biotin binding capacity is reported on the product label.

### Protocol for Use of Streptavidin Conjugated Microspheres

1. Dispense streptavidin conjugated microspheres into a microcentrifuge tube and centrifuge at 10,000 x G for 5-6 minutes. Remove and discard the supernatant. Resuspend the streptavidin conjugated microspheres in PBS / BSA binding buffer. Repeat three times to wash the microspheres. After the last wash, the microspheres can be resuspended to any volume, however higher concentrations usually work better (at least  $5 \times 10^8$  particles/ml).
2. Incubate the biotinylated antibody with the streptavidin conjugated microspheres from Step 1 for 30 minutes at 4°C. In general, using 15-40 $\mu\text{g}$  of biotinylated antibody/mg of microspheres will effectively coat the microspheres. The researcher is strongly encouraged to optimize the antibody / microsphere ratio prior to using the microspheres in their applications.
3. Centrifuge for 5-6 minutes and discard the supernatant. Resuspend the microspheres in PBS / BSA binding buffer.
4. Repeat Step 3, three times. The microspheres are now ready for use in cell sorting or immunoprecipitation applications.

### Biotin Conjugated Microspheres

Both plain and YG biotin conjugated microspheres are available in a 2 $\mu\text{m}$  diameter. The streptavidin binding capacity is reported on the product label.

### Protocol for Use of Biotin Conjugated Microspheres

1. Dispense biotin conjugated microspheres into a microcentrifuge tube and centrifuge at 10,000 x G for 5-6 minutes. Remove and discard the supernatant. Resuspend the biotin conjugated microspheres in PBS / BSA binding buffer. Repeat three times to wash the microspheres. After the last wash, the microspheres can be resuspended to any volume, however higher concentrations usually work better (at least  $5 \times 10^8$  particles/ml).
2. Incubate the streptavidin labeled component with the biotin conjugated microspheres from Step 1 for 30 minutes at 4°C. In general, using 200-600 $\mu\text{g}$  of streptavidin labeled component/mg of

microspheres will effectively coat the microspheres. The researcher is strongly encouraged to optimize the antibody / microsphere ratio prior to using the microspheres in their applications.

3. Centrifuge for 5-6 minutes and discard the supernatant. Resuspend the microspheres in PBS / BSA binding buffer.
4. Repeat Step 3, three times. The microspheres are now ready for use in cell sorting or immunoprecipitation applications.

**STORAGE AND SAFETY**

**Storage** Store at 4°C. Freezing may result in irreversible aggregation and loss of binding activity.

**Safety** This particle suspension contains sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azides. Upon disposal of material, flush with a large volume of water to prevent azide accumulation. Please consult the Safety Data Sheet for more information.

**These products are for research use only and are not intended for use in humans or for *in vitro* diagnostic use.**

**ORDERING INFORMATION**

Cat. #	Description	Size
24161-1	Streptavidin Fluoresbrite® YG Microspheres, 1.0µm	1ml
24161-5	Streptavidin Fluoresbrite® YG Microspheres, 1.0µm	5ml
24162-1	Streptavidin Microspheres, 1.0µm	1ml
24162-5	Streptavidin Microspheres, 1.0µm	5ml
24159-1	Streptavidin Fluoresbrite® YG Microspheres, 2.0µm	1ml
24159-5	Streptavidin Fluoresbrite® YG Microspheres, 2.0µm	5ml
24160-1	Streptavidin Microspheres, 2.0µm	1ml
24160-5	Streptavidin Microspheres, 2.0µm	5ml
24157-1	Streptavidin Fluoresbrite® YG Microspheres, 6.0µm	1ml
24157-5	Streptavidin Fluoresbrite® YG Microspheres, 6.0µm	5ml
24158-1	Streptavidin Microspheres, 6.0µm	1ml
24158-5	Streptavidin Microspheres, 6.0µm	5ml
24172-1	Biotin Microspheres, 2.0µm	1ml
24172-5	Biotin Microspheres, 2.0µm	5ml
24173-1	Biotin Fluoresbrite® YG Microspheres, 2.0µm	1ml
24173-5	Biotin Fluoresbrite® YG Microspheres, 2.0µm	5ml

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 In Germany Call: +(49) 06201-845200  
 In Germany Fax: +(49) 06201-8452020  
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