

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 approximately 1.25%. They are packaged in a 0.02M Sodium Phosphate buffer (pH 7.4) containing 8mg/ml NaCl, 10 mg/ml Bovine Serum Albumin, 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 the binding capacity.

Material Required

100ml PBS/BSA Binding Buffer

Procedure

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

Preparation of Solution

PBS/BSA Binding Buffer: First, prepare 0.1M phosphate buffer (pH 7.4) by adding 0.1M NaH₂PO₄ to 0.1M Na₂HPO₄, until pH reaches 7.4. Place 20ml of the 0.1M phosphate buffer (pH 7.4) in a 100ml graduated cylinder. Add 0.88g NaCl and 1g Bovine Serum Albumin (BSA) and make up the volume to 100ml. Check the pH of the final solution. If necessary, adjust the 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 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 an interaction of high affinity and specificity. The Streptavidin-Biotin complex is stable over a wide range of pH and temperatures. Both plain and fluorescent yellow-green (YG) Streptavidin Conjugated Microspheres are available in 1 μm , 2 μm , and 6 μm diameters. The biotin binding capacity is reported on the product label.

Protocol for Attachment of Biotinylated Antibodies

1. Dispense Streptavidin Conjugated Microspheres into a microcentrifuge tube and centrifuge at 10,000xG 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 streptavidin microspheres can be resuspended to any volume, however higher concentrations usually work better (at least 5×10^8 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 μg 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 fluorescent yellow-green (YG) Biotin Conjugated Microspheres are available in a 2µm diameter. The streptavidin binding capacity is reported on the product label.

Protocol for Use of Biotin Coated Microspheres

1. Dispense Biotin Conjugated Microspheres into a microcentrifuge tube and centrifuge at 10,000xG 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 5×10^8 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 200–600µg of streptavidin labeled component/mg of microspheres will effectively coat the particles. 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 Stability

This product is stable until the expiration date indicated on the container when stored at 4°C. Freezing may result in irreversible aggregation and loss of binding activity.

This product is for research use only and is not intended for use in humans or for *in vitro* diagnostic use.

Ordering Information

Cat. #	Description	Sizes
Streptavidin Coated Microspheres		
24161	1µm fluorescent (YG), 1.25% solids	1ml, 5ml
24162	1µm undyed, 1.25% solids	1ml, 5ml
24159	2µm fluorescent (YG), 1.25% solids	1ml, 5ml
24160	2µm undyed, 1.25% solids	1ml, 5ml
24157	6µm fluorescent (YG), 1.25% solids	1ml, 5ml
24158	6µm undyed, 1.25% solids	1ml, 5ml
Biotin Conjugated Microspheres		
24172	2µm undyed, 1.25% solids	1ml, 5ml
24173	2µm fluorescent (YG), 1.25% solids	1ml, 5ml

To Order

In The U.S. Call: 1-800-523-2575 • 215-343-6484
 In The U.S. FAX: 1-800-343-3291 • 215-343-0214

In Germany Call: (49) 6221-765767
 In Germany FAX: (49) 6221-764620

Order online anytime at www.polysciences.com.

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