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Protein Conjugated Microspheres

Description

Polysciences, Inc. offers antibodies, Protein A, and Protein G covalently coupled to dyed (fluorescent and non-fluorescent) and undyed microspheres. Antibody conjugated microspheres are used to detect trace amounts of antigens in solution via bead-based ELISA and agglutination tests. Microspheres conjugated with Protein A and Protein G can be used to purify IgG by binding to the Fc portion of antibodies raised in most mammals.

Our protein conjugated microspheres have a diameter of 1.0µm and 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, 10mg/ml bovine serum albumin, 0.1% sodium azide, and 5% glycerol. Each lot of the protein conjugated microspheres is quality control tested for mean particle diameter, % solids, and the concentration of protein conjugated to the microspheres. This information is reported on the product label. The fluorescent yellow-green (YG) microspheres have an excitation maximum of 445nm and an emission maximum of 500nm, similar to FITC. For best results, these microspheres should be stored at 4˚C, not frozen, and mixed before using.

Procedure

These protocols are offered as guides. Specific situations may require one or more alterations to these protocols. Researchers are advised to optimize the use of particles in any application.

Antibody Conjugated Microspheres

Goat anti-Mouse IgG (H&L) and Goat anti-Rabbit IgG (H&L) are available on 1.0µm polystyrene microspheres. These antibodies are available on plain, visible blue dyed, or fluorescent yellow-green (YG) microspheres. The antibody concentration is 250-350µg/ml depending on the lot.

Protocol for Use with Antibody Conjugated Microspheres

1. In order to remove the sodium azide and transfer to the appropriate buffer, the microspheres must be washed before use. To do this, aliquot 0.5ml of 1.25% Antibody Conjugated Microspheres into an Eppendorf centrifuge tube (1.5-1.9ml capacity). Add sufficient amount of buffer or cell culture media to fill the tube. *Note:* Use buffer or culture media that is compatible with the current application. Centrifuge in a micro-centrifuge at 10,000 x G for 5-6 minutes. Carefully remove the supernatant using a Pasteur pipette. Discard supernatant and resuspend microspheres in fresh buffer. Repeat this procedure three times in order to sufficiently wash the microspheres.
2. Incubate the microspheres with an appropriate amount of the target antigen for a minimum of 20-30 minutes at 4˚C. This can also be performed at room temperature, if necessary. The amount of target antigen can vary widely. The researcher is strongly encouraged to optimize the antibody/microsphere ratio prior to using the microspheres in their applications. Antigen amounts may range from 10-300µg.
3. To remove any excess antigen that is not attached to the microspheres, wash the microspheres one time using the centrifugation procedure outlined in Step 1. The microsphere-antigen complex is now ready for analysis or experimentation.

Protein A and Protein G Conjugated Microspheres

Protein A and Protein G are available on 1.0µm polystyrene microspheres. These proteins are available on plain, visible blue dyed, or fluorescent yellow-green (YG) microspheres. The protein concentration is 150-250µg/ml depending on the lot.

 Rev. #004, Active: 10/July/2008

Protocol for Use with Protein A and Protein G Conjugated Microspheres
Protein A/G Buffer: 0.1M Tris-HCl, 0.15M NaCl

1. Add 100µl of Protein A or Protein G microspheres to a 1.5ml microcentrifuge tube.
2. Wash microspheres three times with 750µl of Protein A/G Buffer by mixing the buffer, centrifuging in a micro-centrifuge for 5-6 minutes at 10,000 x G, and then removing the supernatant.
3. To the washed microspheres, add 50µl of serum containing the target IgG and 50µl of Protein A/G Buffer. Incubate for 1 hour at 4˚C, vortexing every five minutes.
4. Centrifuge microspheres 5-6 minutes at 10,000 x G in a micro-centrifuge. Remove the supernatant and discard.
5. Wash with 750µl of Protein A/G Buffer three times.
6. Add 50µl of 0.1M glycine, pH 2.5, vortex, and incubate for five minutes. Centrifuge to separate the microspheres and save supernatant, which contains the purified IgG.

Storage and Stability
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 Material Safety Data Sheet for more information.

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	Size	Cat. #	Description	Size
	Goat anti-Mouse IgG (H&L)			**Protein A**	
17697-1	Blue Dyed Polystyrene Microspheres	1ml	17699-1	Blue Dyed Polystyrene Microspheres	1ml
17694-1	Undyed Microspheres	1ml	17698-1	Undyed Microspheres	1ml
17843-1	Fluoresbrite® YG Polystyrene Microspheres	1ml	17845-1	Fluoresbrite® YG Polystyrene Microspheres	1ml
	Goat anti-Rabbit IgG (H&L)			**Protein G**	
17696-1	Blue Dyed Polystyrene Microspheres	1ml	21105-1	Blue Dyed Polystyrene Microspheres	1ml
17693-1	Undyed Microspheres	1ml	21106-1	Undyed Microspheres	1ml
17844-1	Fluoresbrite® YG Polystyrene Microspheres	1ml	21107-1	Fluoresbrite® YG Polystyrene Microspheres	1ml

To Order
In The U.S. Call: 1-800-523-2575 • 215-343-6484
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Order online anytime at **www.polysciences.com.**

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