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TECHNICAL DATA SHEET 615

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Protein Conjugated Microspheres

INTRODUCTION

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 1.0 μ m 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-Rabbit IgG (H&L) are available on 1.0 μ m polystyrene microspheres. These antibodies are available on plain, visible blue dyed, or fluorescent yellow-green (YG) microspheres.

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 10-300 μ g.

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 1.0 μ m polystyrene microspheres. These proteins are available on plain, visible blue dyed, or fluorescent yellow-green (YG) microspheres.

Protocol for Use with Protein A and Protein G Conjugated Microspheres

Protein A/G Buffer: 0.1M Tris-HCl, 0.15M NaCl

1. Add 100 μ l of Protein A or Protein G microspheres to a 1.5ml microcentrifuge tube.
2. Wash microspheres three times with 750 μ l 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 50 μ l of serum containing the target IgG and 50 μ l 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 microcentrifuge. Remove the supernatant and discard.
5. Wash with 750 μ l of Protein A/G Buffer three times.
6. To elute the bound antibody, add 50 μ l 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 SAFETY

Storage Store at 4-8°C. Freezing of particles 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
	Goat anti-Mouse IgG (H&L)	
17697	Blue Dyed Polystyrene Microspheres	1ml
17843	Fluoresbrite® YG Polystyrene Microspheres	1ml
	Goat anti-Rabbit IgG (H&L)	
17693	Undyed Microspheres	1ml
17696	Blue Dyed Polystyrene Microspheres	1ml
17844	Fluoresbrite® YG Polystyrene Microspheres	1ml
	Protein A	
17698	Undyed Microspheres	1ml
17699	Blue Dyed Polystyrene Microspheres	1ml
17845	Fluoresbrite® YG Polystyrene Microspheres	1ml
	Protein G	
21106	Undyed Microspheres	1ml
21105	Blue Dyed Polystyrene Microspheres	1ml
21107	Fluoresbrite® YG Polystyrene Microspheres	1ml

TO ORDER

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