Polysciences, Inc.

*Corporate Headquarters*
*400 Valley Road*
*Warrington, PA 18976*
*1-800-523-2575*
*FAX 1-800-343-3291*
*Email: info@polysciences.com*
*www.polysciences.com*

*Europe - Germany*
*Polysciences Europe GmbH*
*Handelsstr. 3*
*D-69214 Eppelheim, Germany*
*(49) 6221-765767*
*FAX (49) 6221-764620*
*Email: info@polysciences.de*



# *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 & 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 max of 445nm and an emission max of 500nm, similar to FITC. For best results, these microspheres should be stored at 4°C, not frozen, and mixed before using.

***These products are for research use only, not intended for use in humans or in vitro diagnostics use.***

## 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:**
*(This protocol is offered as a guide. Specific situations may require one or more alterations of this protocol.)*

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. (Use buffer or culture media that is compatible with the current application.) Centrifuge in a micro-centrifuge at 10,000xG for 5-6 minutes. Carefully remove the supernatant using a Pasteur pipette. Discard supernatant and resuspend microspheres in fresh buffer. Repeat this procedure 3 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) dyed microspheres. The Protein concentration is 150-250µg/ml depending on the lot.

**Protocol for Use:**
*(This protocol is offered as a guide. Specific situations may require one or more alterations of this protocol.)*

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 with buffer, centrifuging in a micro-centrifuge for 5-6 minutes at 10,000xG and 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 10,000xG in a micro-centrifuge. Remove the supernatant and discard.

5. Wash microspheres 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.

**Ordering Information:**

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

**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
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Order online anytime at **www.polysciences.com**