Product Data Sheet 854

Flow Cytometry Protein A and Protein G Antibody Binding Beads

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Bangs Laboratories, Inc.

BEADS ABOVE THE REST™

DESCRIPTION

Single population Protein A or Protein G microspheres are suitable for labeling with conjugated antibodies from a range of hosts. Labeled microspheres may be used as single-population reference standards or in conjunction with an unlabeled population for compensation purposes.

Typical affinities of Proteins A and G for immunoglobulins from different host species and for different subclasses follow. Because Protein A and Protein G specificities and affinities for IgG vary, investigators are encouraged to research or test specific antibodies as needed.

Table 1: Affinities for Antibodies from Various Species

Host Species	Antibody Class	Protein A	Protein G
Goat	Total IgG	W	S
	IgG1	W	S
	IgG2	S	S
Rabbit	Total IgG	S	S
Hamster	Total IgG	M	W
Sheep	Total IgG	W	S
	IgG1	W	S
	IgG2	S	S
Guinea Pig	Total IgG	S	W
Donkey	Total IgG	M	S

W = weak, M = moderate, S = strong

Though Protein A and G microspheres may be used for mouse, rat, and human antibodies as appropriate, Bangs Laboratories offers dedicated binding standards for antibodies from these host species.

CHARACTERISTICS

Mean Diameter: 7-9µm
Particle Concentration: 2 x 10^6 microspheres/mL
Volume: 1mL (20 tests); 5mL (100 tests); 14mL (280 tests)

MATERIAL

Material Supplied

- Flow Cytometry Protein A or Protein G Antibody Binding Beads

Material Required

- Fluorochrome-labeled antibodies
- Cell samples
- Suspension solution
- Sample test tubes
- Vortex mixer
- Flow cytometer

PROCEDURE

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

Bead Labeling

Prepare a separate sample of Protein A or Protein G Antibody Binding Beads for each fluorochrome-labeled antibody as follows:

1. Place one drop (~50µL, ~100,000 beads) of Antibody Binding Beads into a test tube. Add the fluorochrome-conjugated antibody that is being used for cell labeling. You may use the amount suggested by the antibody supplier for cell labeling, or more or less to achieve saturation. An antibody titration may be performed if necessary.
2. Incubate for 30 minutes with occasional agitation.
3. Wash 2 times and resuspend in 500µL of the suspension solution. *Note:* To wash, centrifuge at low speed (<2000 RPM) for 2 minutes.

Labeled microspheres may be used as a general reference standard, or for specific tasks such as compensation. Labeled microspheres should be prepared immediately prior to use. Affinity interactions are of variable strength, and antibody transfer between populations could occur if microspheres are mixed with other unlabeled or labeled bead populations.

Compensation: Data Collection and Instrument Adjustment

1. Perform routine set up of the analysis range (adjustment of PMT settings).
2. Perform compensation adjustments of each fluorescence channel separately.
3. Compensation
   a. For software compensation, collect 10,000 events in a list mode file without gates and with the compensation circuits off. Gate the singlet population in the list mode files and make the appropriate adjustments in the software to make the 2 populations have equal intensities in the secondary fluorescence detectors.
   b. For hardware compensation, gate on the singlet population and, while running, adjust the compensation circuits such that the 2 populations have equal intensities in the secondary fluorescence detectors.
   c. *Alternative Hardware Compensation:* After washing, samples of the microspheres labeled with different antibodies may be mixed together and analyzed. However, adjustments to the compensation circuits should be performed within an hour of mixing.

4. Validate compensation settings with cells labeled with the same conjugated antibodies.

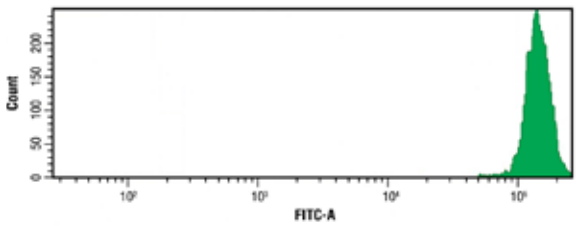
Labeled microspheres may be used in conjunction with unlabeled Protein A or Protein G populations or a Certified Blank™ population for compensation purposes. Affinity interactions are of variable strength, and antibody transfer between populations could occur if microspheres are mixed with other unlabeled or labeled bead populations.

Recommendations

For consistency of data across instruments and time, it is recommended that a unified analysis range (Unified Window of Analysis) be used. The Unified Window of Analysis may be achieved by setting the PMT's of the detectors with Bangs' Right Reference Standard™ or QC Windows® when performing your daily set-up.

Expected Values

Figure 1 shows a histogram of Flow Cytometry Protein G Antibody Binding Beads labeled with human IgG-FITC.



NOTES

If poor results are encountered with a specific run, stain and run a fresh sample. If results are still sub-optimal, you may:

- Drain and fill the flow cell several times to eliminate air bubbles and debris.
- Wash fluidics system by running a fresh solution of 10% household bleach. Follow manufacturer's instructions.
- Check system for pressure leaks.
- Check the properties of diluent and sheath fluid (such as pH).
- Check alignment of the instrument.
- Consult your service engineer.

REFERENCES

1. **Schwartz, A., E. Fernandez-Repollet.** 1993. Development of clinical standards for flow cytometry. *Ann NY Acad Sci,* 677: 28-39.
2. **Shapiro, H.M.** 1995. *Practical flow cytometry, 3rd ed.* New York: Wiley-Liss, Inc.
3. **Schwartz, A., G.E. Marti, R. Poon, J.W. Gratama, E. Fernandez-Repollet.** 1998. Standardizing flow cytometry: a classification system of fluorescence standards used for flow cytometry. *Cytometry*, 33(2):106-114.

TRADEMARKS AND REGISTERED TRADEMARKS

1. Certified Blank™, QC Windows®, Quantum™, Right Reference Standard™, and Simply Cellular® are trademarks or registered trademarks of Bangs Laboratories, Inc.
2. Cy™, including Cy5, is a trademark of GE Healthcare Limited. These products are manufactured under license from Carnegie Mellon University under U.S. Patent Number 5,268,486 and related patents.

STORAGE AND STABILITY

Store at 2-8°C. Freezing may result in irreversible aggregation and loss of binding activity. Stable for 12 months from date of purchase, provided the product is handled in accordance with the manufacturer's recommendations. The reagent should be kept in its original bottle.

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.

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

ORDERING INFORMATION

Cat. Code	Description	Size
553	Flow Cytometry Protein A Antibody Binding Beads	1mL, 5mL, or 14mL
554	Flow Cytometry Protein G Antibody Binding Beads	1mL, 5mL, or 14mL

RELATED PRODUCTS

Cat. Code	Description	Sizes
890	Certified Blank™ Reference Standard	1mL, 5mL, or 14mL
847	QC Windows® (FITC/PE/PE-Cy™5)	1mL, 5mL, or 14mL
815	Quantum™ Simply Cellular® anti-Mouse IgG	1mL, 5mL, or 14mL
816	Quantum™ Simply Cellular® anti-Human IgG	1mL, 5mL, or 14mL
817	Quantum™ Simply Cellular® anti-Rat IgG	1mL, 5mL, or 14mL
810	Simply Cellular® anti-Mouse IgG	1mL, 5mL, or 14mL
812	Simply Cellular® anti-Human IgG	1mL, 5mL, or 14mL
813	Simply Cellular® anti-Rat IgG	1mL, 5mL, or 14mL

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