

AbC™ Anti-Mouse Bead Kit

Catalog no. A10344

Table 1. Contents and storage information.

Material	Amount	Composition	Storage*	Stability
AbC™ capture beads (Component A)	5 mL	3 × 10 ⁶ beads/mL in phosphate buffered saline (PBS) with 0.1% BSA and 2 mM sodium azide	<ul style="list-style-type: none"> • 2–6°C • DO NOT FREEZE 	When stored as directed, this kit is stable for at least 1 year.
Negative beads (Component B)	5 mL	3 × 10 ⁶ beads/mL in deionized water containing 2 mM sodium azide and 0.05% Tween® 20		

Number of assays: Sufficient material is supplied for 100 assays based on the protocol below.

Introduction

The AbC™ Anti-Mouse Bead Kit provides a consistent, accurate, and simple-to-use technique for the setting of flow cytometry compensation when using fluorochrome-conjugated mouse antibodies. The kit contains two types of specially modified polystyrene microspheres, the AbC™ capture beads, that bind all isotypes of mouse immunoglobulin, and the negative beads that have no antibody binding capacity.

After incubation with a fluorochrome-conjugated mouse antibody, the two bead components provide distinct positive and negative populations of beads that you can use to set compensation (Figure 1). You can perform compensation with the same fluorochrome-labeled antibody used for cell staining. Because of the consistent nature of bead scatter and high surface antibody binding capacity, this allows you to more consistently and accurately set compensation for any combination of fluorochrome-labeled mouse antibodies.

The AbC™ capture beads and negative beads have a diameter of approximately 6 μm (actual size for each lot is listed on the component vial). The bead suspensions are supplied in dropper vials for convenient sample application.

Before Starting

Materials Required but Not Provided

- Mouse antibody conjugate
- PBS or equivalent

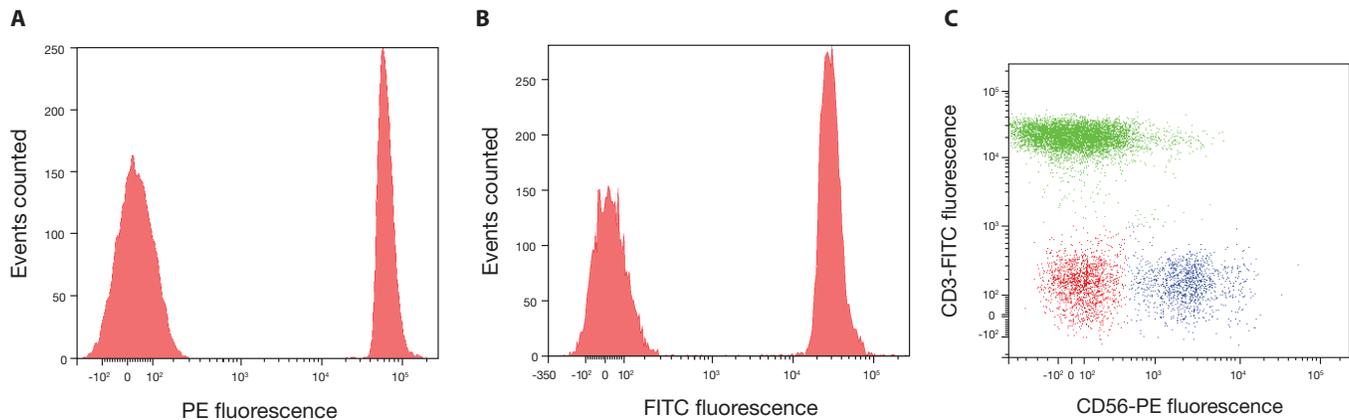


Figure 1. Compensation using the AbC™ Anti-Mouse Bead Kit. (A) Phycoerythrin (PE)-conjugated mouse anti-human CD56 antibody (Invitrogen Cat. no. MHCD56044) labeling the AbC™ capture beads for a positive signal and negative beads providing a negative signal. (B) FITC-conjugated mouse anti-human CD3 (Invitrogen Cat. no. MHCD03014) labeling the AbC™ capture beads for a positive signal and negative beads providing a negative signal. (C) Dual parameter plot showing gated human lymphocytes labeled with Phycoerythrin-conjugated mouse anti-human CD56 and FITC-conjugated mouse anti-human CD3 using compensation settings obtained with the AbC™ Anti-Mouse Bead Kit.

Experimental Protocols

Using the AbC™ Anti-Mouse Bead Kit

- 1.1 Completely resuspend the AbC™ capture beads (Component A) and negative beads (Component B) by gently vortexing for 30 seconds before use.
- 1.2 Label a sample tube for each fluorochrome-conjugated mouse antibody you are using, and add 1 drop of AbC™ capture beads (Component A) to each tube.
- 1.3 Add a pre-titrated amount of each mouse antibody conjugate to the AbC™ capture bead suspension in the designated tube and mix well. Make sure to deposit the antibody directly to the bead suspension.
- 1.4 Incubate for 15 minutes at room temperature, **protected from light**.
- 1.5 Add 3 mL of PBS or other buffer to sample tubes. Centrifuge for 5 minutes at $200 \times g$.
- 1.6 Carefully remove the supernatant from tubes and resuspend the bead pellet by adding 0.5 mL of PBS or other buffer to sample tubes.
- 1.7 Add one drop of negative beads (Component B) to the tubes and mix well.
- 1.8 Vortex tubes before analyzing using flow cytometry. You may briefly sonicate to increase the percentage of singlet beads, if necessary.
- 1.9 Perform manual or automatic compensation according to the preferred procedure for the flow cytometer in use. Gate on the bead singlet population based on FCS and SSC characteristics.

Combining AbC™ and ArC™ Kits

The ArC™ Amine Reactive Compensation Bead Kit is designed to facilitate compensation when using any of the LIVE/DEAD® fixable dead cell stains, all amine-reactive dyes. This kit provides two types of specially modified polystyrene microspheres, the ArC™ reactive beads (Component A) that bind any of the amine-reactive dyes, and the ArC™ negative beads (Component B), that have no reactivity. Using the two kit components with any amine-reactive dye will provide distinct positive and negative populations of beads. You can use the AbC™ Anti-Mouse Bead Kit and the ArC™ Amine Reactive Compensation Bead Kit together to calculate compensation in multicolor immunophenotyping experiments that incorporate a LIVE/DEAD® fixable dye by following the protocol outlined below:

- 2.1. Gently vortex the ArC™ Amine Reactive Beads Kit and the AbC™ Anti-Mouse Bead Kit components for 30 seconds to completely resuspend before use.
- 2.2. Label a sample tube for the amine-reactive dye you are using, and add 1 drop of ArC™ reactive beads (Component A) to a labeled sample tube. Allow ArC™ reactive beads to sit in the tube for 5 minutes to warm to room temperature.
- 2.3. Prepare fluorescent reactive dye according to kit instructions included in the LIVE/DEAD® Fixable Dead Cell Stain Kit. For optimal performance of ArC™ reactive beads, always use freshly prepared amine-reactive dye. Do **not** use previously frozen dye solution.
- 2.4. Add the amount of LIVE/DEAD® fixable dead cell stain listed in Table 2 to the bead suspension and mix well. Make sure to deposit the amine-reactive dye directly to the bead suspension.

Table 2. Amount of amine-reactive LIVE/DEAD® fixable dead cell stain for use with ArC™ reactive beads.

Amine-reactive dye for use with ArC™ reactive beads	Amount
LIVE/DEAD® Fixable Blue stain	3 µL
LIVE/DEAD® Fixable Violet stain	1 µL
LIVE/DEAD® Fixable Aqua stain	3 µL
LIVE/DEAD® Fixable Yellow stain	3 µL
LIVE/DEAD® Fixable Green stain	3 µL
LIVE/DEAD® Fixable Red stain	1 µL
LIVE/DEAD® Fixable Far Red stain	3 µL
LIVE/DEAD® Fixable Near-IR stain	1 µL

- 2.5. Label another sample tube for each fluorochrome-conjugated antibody you are using, and add 1 drop of AbC™ capture beads (Component A in the AbC™ Anti-Mouse Bead Kit) to each tube.
- 2.6. Add a pre-titrated amount of antibody conjugate to the appropriate tube and mix well. Make sure to deposit the antibody directly to the bead suspension.
- 2.7. Incubate for 30 minutes at room temperature, **protected from light**.
- 2.8. Add 3 mL of PBS or other buffer to each sample tube. Centrifuge at 300 × g for 5 minutes to collect the beads.
- 2.9. Carefully remove all supernatant from each tube. If using the red fluorescent reactive dye (Cat. no. L23102), repeat step 8 for that tube.

- 2.10. Resuspend the bead pellet by adding 0.5 mL of buffer to each sample tube.
- 2.11. Add one drop of negative beads (Component B in the AbC™ Anti-Mouse Bead Kit) to sample tube(s) containing the AbC™ capture beads.
- 2.12. Add one drop of ArC™ negative beads (Component B in the ArC™ Amine Reactive Compensation Bead Kit) to sample tube(s) containing the ArC™ reactive beads.
- 2.13. Vortex tubes before analyzing using flow cytometry.
- 2.14. Perform manual or automatic compensation according to the preferred procedure for the flow cytometer in use. Gate on the bead singlet population based on FSC and SSC characteristics.

Product List Current prices may be obtained from our website or from our Customer Service Department.

Cat. no.	Product Name	Unit Size
A10344	AbC™ Anti-Mouse Bead Kit *for mouse antibody capture* *for flow cytometry* *100 tests*	1 kit
<i>Related Products</i>		
A10346	ArC™ Amine Reactive Compensation Bead Kit *for use with amine reactive dyes* *for flow cytometry compensation* *100 tests* ..	1 kit
L10119	LIVE/DEAD® Fixable Near-IR Dead Cell Stain Kit *for 633 or 635 nm excitation* *200 assays*	1 kit
L10120	LIVE/DEAD® Fixable Far Red Dead Cell Stain Kit *for 633 or 635 nm excitation* *200 assays*	1 kit
L23101	LIVE/DEAD® Fixable Green Dead Cell Stain Kit *for 488 nm excitation* *200 assays*	1 kit
L23102	LIVE/DEAD® Fixable Red Dead Cell Stain Kit *for 488 nm excitation* *200 assays*	1 kit
L23105	LIVE/DEAD® Fixable Blue Dead Cell Stain Kit *for UV excitation* *200 assays*	1 kit
L34955	LIVE/DEAD® Fixable Violet Dead Cell Stain Kit *for 405 nm excitation* *200 assays*	1 kit
L34957	LIVE/DEAD® Fixable Aqua Dead Cell Stain Kit *for 405 nm excitation* *200 assays*	1 kit
L34959	LIVE/DEAD® Fixable Yellow Dead Cell Stain Kit *for 405 nm excitation* *200 assays*	1 kit
L34960	LIVE/DEAD® Fixable Dead Cell Stain Sampler Kit *for flow cytometry* *320 assays*	1 kit
10010-049	Phosphate Buffered Saline (PBS) 7.2 (1X), liquid	10 × 500 mL
20012-050	Phosphate Buffered Saline (PBS) 7.4 (1X), liquid	10 × 500 mL

Contact Information

Molecular Probes, Inc.

29851 Willow Creek Road
Eugene, OR 97402
Phone: (541) 465-8300
Fax: (541) 335-0504

Customer Service:

6:00 am to 4:30 pm (Pacific Time)
Phone: (541) 335-0338
Fax: (541) 335-0305
probesorder@invitrogen.com

Toll-Free Ordering for USA:

Order Phone: (800) 438-2209
Order Fax: (800) 438-0228

Technical Service:

8:00 am to 4:00 pm (Pacific Time)
Phone: (541) 335-0353
Toll-Free (800) 438-2209
Fax: (541) 335-0238
probestech@invitrogen.com

Invitrogen European Headquarters

Invitrogen, Ltd.
3 Fountain Drive
Inchinnan Business Park
Paisley PA4 9RF, UK
Phone: +44 (0) 141 814 6100
Fax: +44 (0) 141 814 6260
Email: euroinfo@invitrogen.com
Technical Services: eurotech@invitrogen.com

**For country-specific contact information,
visit www.invitrogen.com.**

Further information on Molecular Probes products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Paisley, United Kingdom. All others should contact our Technical Service Department in Eugene, Oregon.

Molecular Probes products are high-quality reagents and materials intended for research purposes only. These products must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Please read the Material Safety Data Sheet provided for each product; other regulatory considerations may apply.

Limited Use Label License No. 223: Labeling and Detection Technology

The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) to not transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. Invitrogen Corporation will not assert a claim against the buyer of infringement of the above patents based upon the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine or prophylactic product developed in research by the buyer in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product. If the purchaser is not willing to accept the limitations of this limited use statement, Invitrogen is willing to accept return of the product with a full refund. For information on purchasing a license to this product for purposes other than research, contact Molecular Probes, Inc., Business Development, 29851 Willow Creek Road, Eugene, OR 97402, Tel: (541) 465-8300. Fax: (541) 335-0354.

Several Molecular Probes products and product applications are covered by U.S. and foreign patents and patents pending. All names containing the designation ® are registered with the U.S. Patent and Trademark Office.

Copyright 2009, Molecular Probes, Inc. All rights reserved. This information is subject to change without notice.