molecular probes
life technologies

AbC™ Total Antibody Compensation Bead Kit

Catalog nos. A10497, A10513

Table 1. Contents and storage

Material	Amount		Composition	Storage	Stability
	A10497	A10513			
AbC™ Total Compensation capture beads (Component A)	5 mL	1.25 mL	3×10^6 beads/mL in phosphate buffered saline (PBS) with 0.1% BSA and 2 mM sodium azide	• 2–8°C • DO NOT FREEZE	When stored as directed, this kit is stable for at least 1 year.
Negative beads (Component B)	5 mL	1.25 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™ Total Antibody Compensation Bead Kit provides a consistent, accurate, and simple-to-use technique for the setting of flow cytometry compensation when using fluorochrome-conjugated antibodies. The kit contains two types of specially modified polystyrene microspheres, the AbC™ Total Compensation capture beads, that bind all isotypes of mouse, rat, hamster, and rabbit immunoglobulin, and the negative beads that have no antibody binding capacity (Figure 1, page 2).

After incubation with a fluorochrome-conjugated antibody, the two bead components provide distinct positive and negative populations of beads that you can use to set compensation (Figure 2, page 2). 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 antibodies.

The AbC™ Total Compensation 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.

Figure 1. Histograms showing the staining of the AbC™ Total Antibody Compensation Bead Kit. The histograms show the signal separation of the positive capture beads from negative beads after binding to mouse (top left), rat (top right), and hamster (bottom left) monoclonal antibodies, and rabbit (bottom right) mono- and polyclonal antibodies. Capture beads were labeled with an optimized amount of each PE antibody conjugate and analyzed on an Attune® Acoustic Focusing Cytometer using 488-nm excitation and a 574/26-nm BP filter.

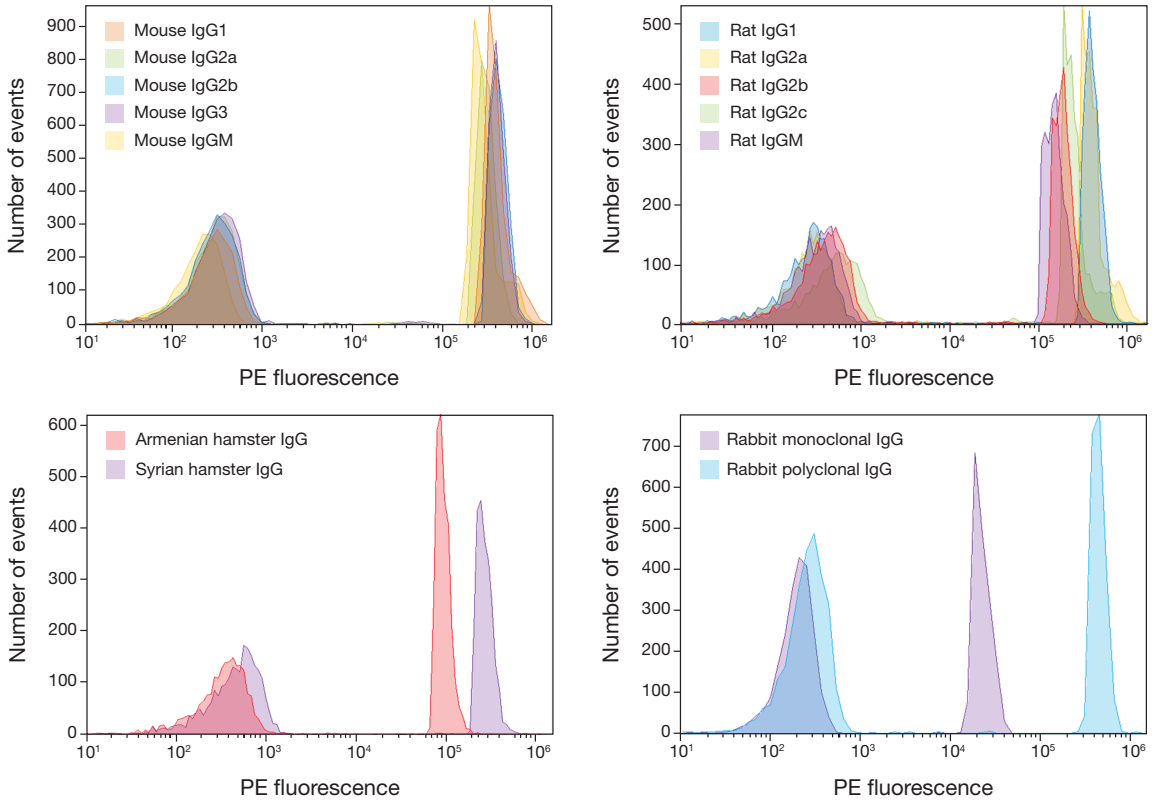
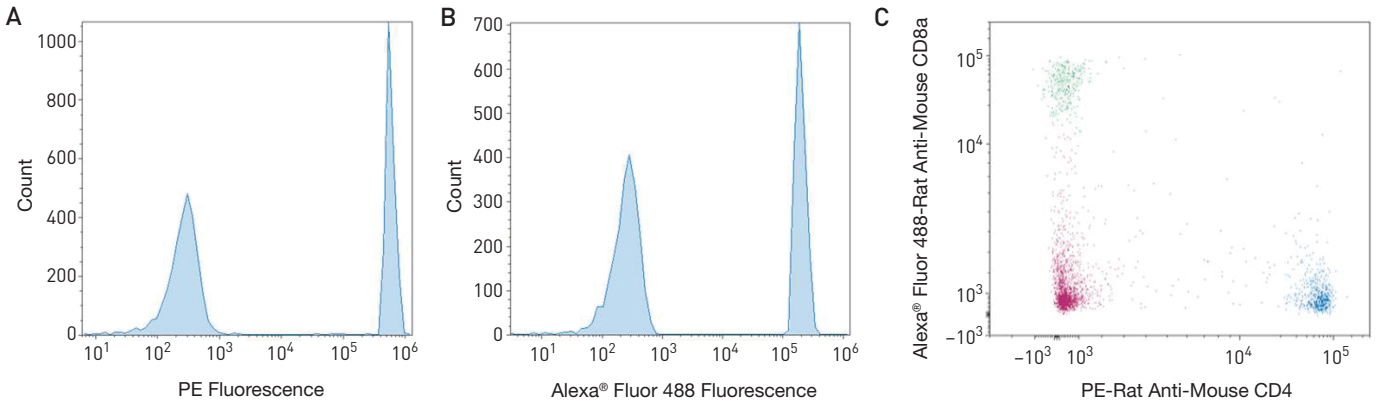


Figure 2. Compensation using the AbC™ Total Antibody Compensation Bead Kit. **(A)** The AbC™ Total capture beads bound to phycoerythrin (PE)-conjugated rat anti-mouse CD4 antibodies (Cat. no. MCD0404) generated positive signal in the BL2 channel (575/26) and unconjugated beads provided the negative signal. **(B)** The Total AbC™ capture beads bound to Alexa Fluor® 488-conjugated rat anti-mouse CD8a (Cat. no. MCD0820) generated positive signal in the BL1 channel (530/30) and unconjugated beads provided the negative signal. **(C)** A dual-parameter dot plot showed gated mouse splenocytes labeled with both phycoerythrin-conjugated rat anti-mouse CD4 and Alexa Fluor® 488-conjugated rat anti-mouse CD8a using compensation settings obtained with the AbC™ Total Antibody Compensation Bead Kit.



Before Starting

Materials Required but Not Provided

- Mouse, Rat, Hamster, or Rabbit antibody conjugate
- PBS or equivalent

Experimental Protocols

Using the AbC™ Total Antibody Compensation Bead Kit

1.1 Completely resuspend the AbC™ Total Compensation capture beads (Component A) and negative beads (Component B) by gently vortexing for 10 seconds before use.

1.2 Label a sample tube for each fluorochrome-conjugated antibody you are using, and add 1 drop of AbC™ Total Compensation capture beads (Component A) to each tube.

1.3 Add a pre-titrated amount of each mouse antibody conjugate to the AbC™ Total Compensation 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 250 × *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.

The ArC™ Amine Reactive Compensation Bead Kit (Cat. no. A10346) 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™ Total Antibody Compensation 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.

To streamline the protocol when combining the two kits, you can substitute the negatives beads (Component B) in the AbC™ Total Antibody Compensation Bead Kit for the negative beads (Component B) of the ArC™ Amine Reactive Compensation Bead Kit.

2.1. Gently vortex the ArC™ Amine Reactive Beads Kit and the AbC™ Total Antibody Compensation Bead Kit components for 10 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 in the ArC™ Amine Reactive Compensation Bead Kit) 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™ Total Compensation capture beads (Component A in the AbC™ Total Antibody Compensation 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 250 × *g* for 5 minutes to collect the beads.

2.9. Carefully remove all supernatant from each tube.

Note: If using the red fluorescent reactive dye (Cat. no. L23102), repeat step 2.8 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™ Total Antibody Compensation Bead Kit) to sample tube(s) containing the AbC™ Total Compensation 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 the 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
A10497	AbC™ Total Antibody Compensation Bead Kit *for flow cytometry* *100 tests*	1 kit
A10513	AbC™ Total Antibody Compensation Bead Kit *for flow cytometry* *25 tests*	1 kit
Related Products		
A10344	AbC™ Anti-Mouse Bead Kit *for mouse antibody capture* *for flow cytometry* *100 tests*	1 kit
A10346	ArC™ Amine Reactive Compensation Bead Kit *for use with amine reactive dyes * *for flow cytometry compensation* *100 tests*	1 kit
A10389	AbC™ anti-Rat/Hamster Bead Kit *for rat/hamster antibody capture* *for flow cytometry* *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) 7.4 (1X), liquid	10 × 500 mL
20012-050	Phosphate Buffered Saline (PBS) 7.2 (1X), liquid	10 × 500 mL

Purchaser Notification

These high-quality reagents and materials must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Read the Safety Data Sheet provided for each product; other regulatory considerations may apply.

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