

ArC™ Amine Reactive Compensation Bead Kit

Catalog no. A10346

Table 1. Contents and storage information.

| Material | Amount | Composition | Storage | Stability |
|-----------------------------------|--------|--|--|--|
| ArC™ reactive beads (Component A) | 5 mL | 8 × 10 ⁶ beads/mL in deionized water | <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. |
| ArC™ 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 ArC™ Amine Reactive Compensation Bead Kit provides a consistent, accurate and simple-to-use technique for the setting of flow cytometry compensation when using any of the LIVE/DEAD® fixable dead cell stains, all amine-reactive dyes. The LIVE/DEAD® Fixable Dead Cell Stain Kits use a novel method to evaluate the viability of mammalian cells by flow cytometry¹⁻⁵. These assays are based on the reaction of a fluorescent dye with cellular amines. The reactive dye can enter the compromised membranes of necrotic cells and react with free amines in the interior and on the surface of the cell, resulting in intense fluorescent staining. In contrast, only the cell-surface amines of viable cells are available to react with the dye, resulting in relatively dim staining. The difference in intensity between the live and dead cell populations is typically greater than 50-fold.

The ArC™ Amine Reactive Compensation Bead Kit includes two types of specially modified polystyrene microspheres to allow easy compensation of the LIVE/DEAD® fixable stains: the ArC™ reactive beads (Component A) which bind any of the amine-reactive dyes, and the ArC™ negative beads (Component B) which have no reactivity. After incubation with any amine-reactive dye, the two kit components provide distinct positive and negative populations of beads that you can use to set compensation. When combined with the AbC™ Anti-Mouse Bead Kit for use with fluorochrome-conjugated mouse antibodies, this allows you to more consistently and accurately set compensation for multicolor immunophenotyping experiments which also incorporate a LIVE/DEAD® fixable dead cell stain.

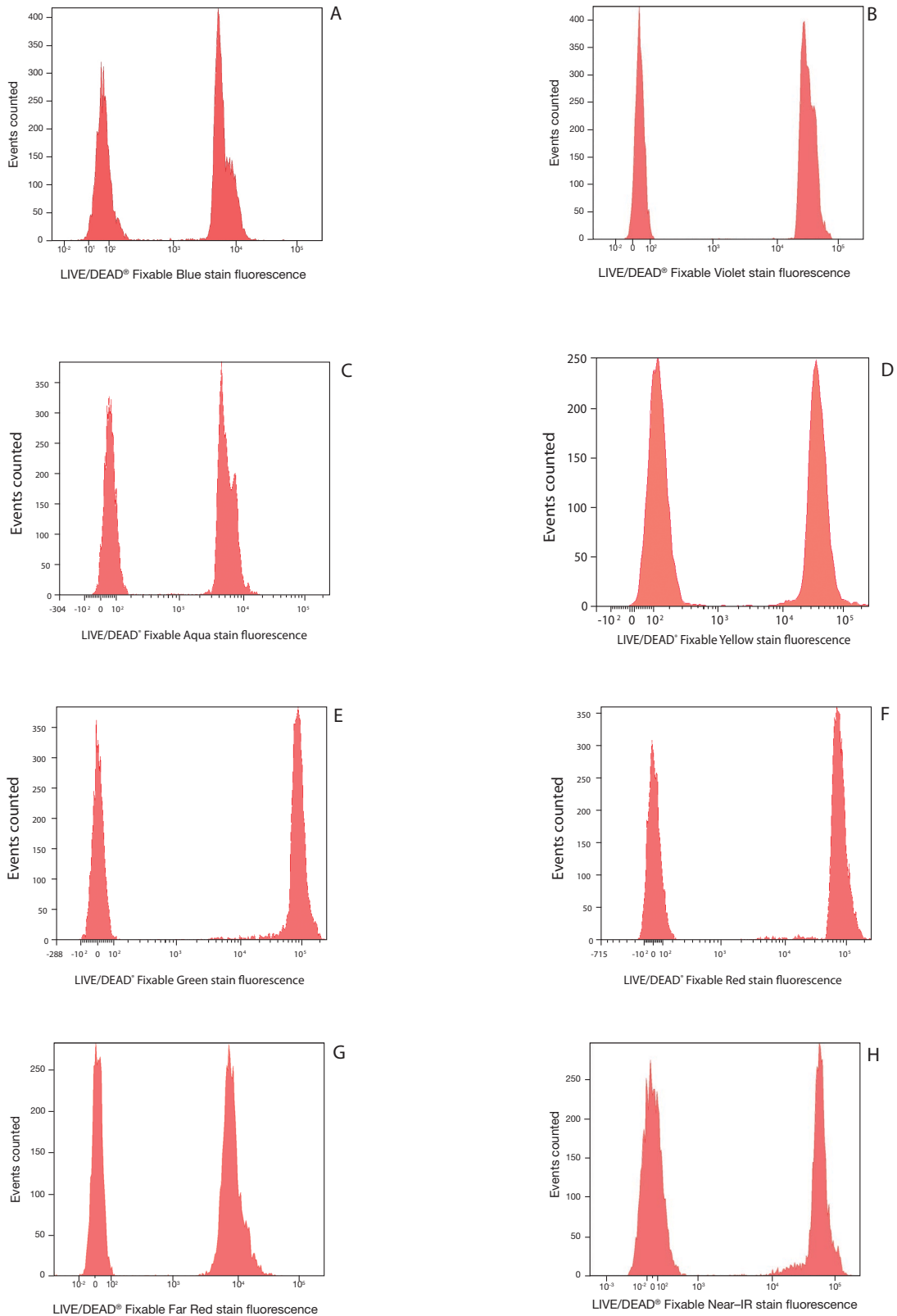


Figure 1. Staining profile of the Arc™ Amine Reactive Compensation Bead Kit components with seven LIVE/DEAD® Fixable Dead Cell Stain Kits. (A) LIVE/DEAD® Fixable Blue dye stained beads were analyzed with UV excitation, emission was collected with a 450/50 nm bandpass filter. (B) LIVE/DEAD® Fixable Violet dye stained beads were analyzed with 405 nm excitation, emission was collected with a 450/50 nm bandpass filter. (C) LIVE/DEAD® Fixable Aqua dye stained beads were analyzed with 405 nm excitation, emission was collected with a 525/50 nm bandpass filter. (D) LIVE/DEAD® Fixable Yellow dye stained beads were analyzed with 405 nm excitation, emission was collected with a 575/26 bandpass filter. (E) LIVE/DEAD® Fixable Green dye stained beads were analyzed with 488 nm excitation, emission was collected with a 525/50 nm bandpass filter. (F) LIVE/DEAD® Fixable Red dye stained beads were analyzed with 488 nm excitation, emission was collected with a 610/20 nm bandpass filter. (G) LIVE/DEAD® Fixable Far Red dye stained beads were analyzed using 633 nm excitation, emission was collected with a 660/20 nm bandpass filter. (H) LIVE/DEAD® Fixable Near-IR dye stained beads were analyzed with 633 nm excitation, emission was collected with a 780/60 nm bandpass filter..

Before Starting

Materials Required but Not Provided

- Buffers for staining (see below)
- LIVE/DEAD® Fixable Dead Cell Stain Kit
- AbC™ Anti-Mouse Bead Kit (if calculating compensation in multicolor immunophenotyping experiments using mouse antibodies)

Buffers for Staining

Buffers appropriate for staining ArC™ reactive beads and cells with a fluorescent amine-reactive dye include phosphate-buffered saline (PBS), Hanks' Balanced Salt Solution (HBSS), and Dulbecco's PBS, without extraneous proteins such as bovine serum albumin or serum. When using an amine-reactive dye, for example the violet fluorescent reactive dye (Cat. no. L34955), avoid using Tris buffers and solutions containing sodium azide or extraneous protein for cell resuspension and washing.

Experimental Protocols

Using ArC™ Amine Reactive Compensation Bead Kit

- 1.1. Gently vortex ArC™ Amine Reactive Compensation Bead Kit components for 30 seconds to completely resuspend before use.
- 1.2. Add 1 drop of ArC™ reactive beads (Component A) to a labeled sample tube.
- 1.3. Allow ArC™ reactive beads to sit in the tube for 5 minutes to warm to room temperature.
- 1.4. Prepare fluorescent amine-reactive dye according to instructions included in the LIVE/DEAD® Fixable Dead Cell Kit. For optimal performance of ArC™ reactive beads, always use freshly prepared amine-reactive dye. Do not use previously frozen dye solution.
- 1.5. Add amount of LIVE/DEAD® fixable dead cell stain listed in Table 2 to the bead suspension and mix well. Make sure the amine-reactive dye is deposited 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 |

- 1.6. Incubate for 30 minutes at room temperature, **protected from light**.
- 1.7. Add 3 mL of PBS or other buffer to sample tube. Centrifuge at $300 \times g$ for 5 minutes.
- 1.8. Carefully remove all the supernatant from tube. If using the red fluorescent reactive dye (Cat. no. L23102), repeat step 7.
- 1.9. Resuspend the bead pellet by adding 0.5 mL of staining buffer to the sample tube.
- 1.10. Add one drop of ArC™ negative beads (Component B) to sample tube. Mix thoroughly.
- 1.11. Vortex tubes before analyzing using flow cytometry.
- 1.12. 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.

Combining ArC™ and AbC™ Kits

The AbC™ Anti-Mouse Bead Kit (Cat. no. A10344) 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 (Component A) that bind all isotypes of mouse immunoglobulin, and negative beads (Component B) that have no antibody binding capacity. After incubating with a fluorochrome-conjugated mouse antibody, the two components provide distinct positive and negative populations of beads that you can use to set compensation. 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 Compensation Bead 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 the 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.
- 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 $300 \times g$ for 5 minutes to collect 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 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.

References

1. J Immunol Methods 313, 199 (2006); 2. J Virol doi:10.1128/JVI.01083 (2008); 3. Stem Cells 26, 1009 (2008); 4. Blood 111, 750 (2008); 5. Blood 111, 3155 (2008).

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

| Cat. no. | Product Name | Unit Size |
|-------------------------|--|-------------|
| A10346 | ArC™ Amine Reactive Compensation Bead Kit *for use with amine reactive dyes **for flow cytometry compensation* *100 tests* . . | 1 kit |
| Related Products | | |
| A10344 | AbC™ Anti-Mouse Bead Kit *for mouse 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 |
| FB001 | IC Fixation Buffer | 100 mL |
| GAS001S-100 | Fixation Medium - Bulk, (MEDIUM A), 1 × 100 mL Fixation Medium *for 1000 tests* | 1000 tests |
| GAS002S-100 | Permeabilization Medium - Bulk, (MEDIUM B), 1 × 100 mL Permeabilization Medium *for 1000 tests* | 1000 tests |
| GAS-003 | Fixation and Permeabilization, 1 × 5 mL *for 50 tests* | 50 tests |
| GAS-004 | Fixation and Permeabilization, 4 × 5 mL *for 200 tests* | 200 tests |
| PB001 | IC Permeabilization Buffer | 2 × 125 mL |
| 10010-049 | Phosphate Buffered Saline (PBS) 7.2 (1X), liquid | 10 × 500 mL |
| 14025-092 | Hanks' Balanced Salt Solution (HBSS) (1X), liquid *contains calcium and magnesium, but no phenol red* | 500 mL |
| 14040-117 | Dulbecco's Phosphate-Buffered Saline (D-PBS) (1X), liquid *contains calcium and magnesium* | 1,000 mL |
| 14040-133 | Dulbecco's Phosphate-Buffered Saline (D-PBS) (1X), liquid *contains calcium and magnesium* | 500 mL |
| 14040-141 | Dulbecco's Phosphate-Buffered Saline (D-PBS) (1X), liquid *contains calcium and magnesium* | 100 mL |
| 14080-055 | Dulbecco's Phosphate-Buffered Saline (D-PBS) (1X), liquid *contains calcium and magnesium, but no phenol red* | 500 mL |
| 14170-112 | Hanks' Balanced Salt Solution (HBSS) (1X), liquid *contains no calcium chloride, magnesium chloride, or magnesium sulfate* | 500 mL |
| 14175-095 | Hanks' Balanced Salt Solution (HBSS) (1X), liquid *contains no calcium chloride, magnesium chloride, magnesium sulfate, or phenol red* | 500 mL |
| 14287-072 | Dulbecco's Phosphate-Buffered Saline (D-PBS) (1X), liquid *contains 1,000 mg/L D-glucose and 36 mg/L sodium pyruvate, calcium, and magnesium, but no phenol red* | 1,000 mL |
| 20012-050 | Phosphate Buffered Saline (PBS) 7.4 (1X), liquid | 10 × 500 mL |
| 24020-117 | Hanks' Balanced Salt Solution (HBSS) (1X), liquid *contains calcium and magnesium* | 500 mL |

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