Product Data Sheet 853

Viability Dye Compensation Standard

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B E A D S ● A B O V E T H E R E S T ™

DESCRIPTION

Viability Dye Compensation Standards are suitable for labeling with LIVE/DEAD® stains or similarly-reactive dyes to generate compensation standards for flow cytometric analyses. Beads are not suitable for labeling with DNA stains such as propidium iodide, DAPI, or SYTOX®, and users should contact Bangs Laboratories for discussion if uncertain as to the compatibility of a specific dye or stain.

Viability Dye Compensation Standards are supplied in a sterile-filtered de-ionized water with surfactant and sodium azide.

CHARACTERISTICS

Mean Diameter: 4µm or 8µm
Particle Concentration: 1 x 10^7 microspheres/mL

MATERIAL

Material Supplied

- Viability Dye Compensation Standard microspheres

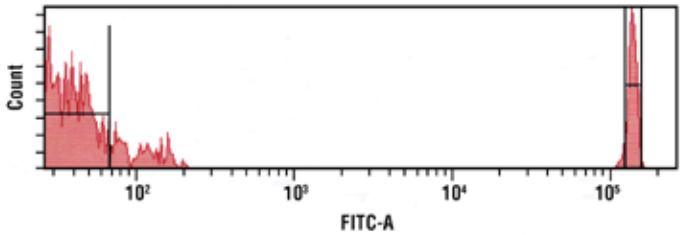
Material Required

- LIVE/DEAD® Viability Dye or similarly-reactive dyes
- PBS, pH 7.4
- Sample tubes
- Pipets
- Rotator
- Vortex mixer
- Flow cytometer

PROCEDURE

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

1. Retrieve the Viability Dye Compensation Standard beads from the refrigerator, and allow them to come to room temperature while end-over-end mixing. Mixing will disrupt small aggregates and ensure a monodisperse suspension.
2. Label a sample tube (1.5mL microcentrifuge tube) and add 1 drop of microsphere suspension (~50µL = ~500,000 beads).
3. Wash the microspheres. Add 0.5mL PBS or other buffer that is free of surfactant and blocker to the sample tube. Centrifuge at 300 x G for 5 minutes. Decant and repeat. Decant and re-suspend using 50µL PBS.
4. Prepare a fresh sample of the fluorescent viability dye (e.g. LIVE/DEAD® or other with similar reactivity) in accordance with the manufacturer's instructions.
5. Add 1-3µL viability stain to the bead suspension and mix well. A titration may be conducted to optimize dye concentration. Cover the sample tube with foil and incubate for 30 minutes.
6. Add 1mL PBS to the sample tube and mix. Centrifuge for 300 x G for 5 minutes, decant, and repeat. Reconstitute beads in PBS or other appropriate buffer.
7. Pulse vortex the bead suspension before running them on the cytometer to achieve highest % singlets.
8. A drop of unstained beads may be used as the blank population, and may be run separately or included with the labeled population.
9. Construct a live gate around singlet population in the FSC/SSC dot plot.
10. Create fluorescence plots for the appropriate detector(s), and perform compensation to achieve desired results.



NOTES

1. To achieve best results, all procedures, including sample preparation and instrument set-up, should be standardized as much as possible.
2. As with other instrument settings, test-specific compensation settings should be established.
3. Dye and / or bead concentrations may be further optimized for best results. Centrifugation force or time may be increased if needed. Incremental adjustments are suggested to avoid aggregation.
4. Compensation bead samples should be protected from light during preparation, and used immediately.

REFERENCES

1. **Perfetto, S.P., P.K. Chattopandhyay, L. Lamoreaux, R. Nguyen, D. Ambrozak, R.A. Koup, M. Roederer.** 2006. Amine reactive dyes: an effective tool to discriminate live and dead cells in polychromatic flow cytometry. *J Immunol Meth*, 313(1-2):199-208.
2. **Shapiro, H.M.** 2003. *Practical flow cytometry, 4th ed.* Hoboken, NJ: John Wiley & Sons (ISBN: 0-471-41125-6).

TRADEMARKS AND REGISTERED TRADEMARKS

1. Simply Cellular® is a registered trademark of Bangs Laboratories, Inc.
2. LIVE/DEAD® and SYTOX® are registered trademarks of Molecular Probes.

STORAGE AND STABILITY

Store at 2-8°C. Do not freeze. Prepared samples may be vortexed briefly if needed to increase the % singlets. Stable for 12 months from date of purchase, provided the product is handled in accordance with the manufacturer's recommendations.

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
450	Viability Dye Compensation Standard, 4µm	3mL
451	Viability Dye Compensation Standard, 8µm	3mL

RELATED PRODUCTS

Cat. Code	Description	Sizes
551	Simply Cellular® anti-Rat Compensation Standard	5mL
552	Simply Cellular® anti-Human Compensation Standard	5mL
820	FITC / PE Compensation Standard	1mL, 5mL, or 14mL

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