eZKine™ 4-Color Compensation Control Kit

Catalog Number: 8822-6856
RUO: For Research Use Only. Not for use in diagnostic procedures.

Product Information

Contents: eZKine™ 4-Color Compensation Control Kit
REF **Catalog Number:** 8822-6856

Temperature Limitation: Store at 2-8°C. Do not freeze. Light-sensitive material.
LOT **Batch Code:** Refer to vial
Use By: Refer to vial
Contains sodium azide

Description

This eZKine™ 4-Color Compensation Control Kit is designed for compensation setup with eZKine™ Whole Blood Intracellular Cytokine Kits. It contains the necessary components to create single-color compensation controls for use with eZKine™ cocktails, including OneComp eBeads and 4 vials of antibody representing each of the 4 fluorochromes used in eZKine™ cocktails. OneComp eBeads can be stained with each of the 4 fluorochrome-conjuated antibodies to set up single-color compensation control tubes.

Components

OneComp eBeads: 1 drop/test, 100 tests
Anti-Human CD3 (SK7) APC: 25 tests
Anti-Human CD4 (SK3) PerCP-eFluor® 710: 25 tests
Anti-Human IL-17A (eBio64DEC17) PE: 25 tests
Anti-Human IFN gamma (4S.B3) FITC: 25 tests

Applications Reported

eZKine™ 4-Color Compensation Control Kit has been reported for use in flow cytometric analysis.

Applications Tested

The components of the eZKine™ 4-Color Compensation Control Kit have been pre-titrated and tested by flow cytometric analysis. The antibodies can be used at 5 µL per test.The OneComp eBeads can be used at 1 drop/test. Refer to the attached protocol for further details.

Related Products

16-0289 eBioscience™ Anti-Human CD28 Functional Grade Purified (CD28.2)
16-0499 eBioscience™ Anti-Human CD49d (Integrin alpha 4) Functional Grade Purified (9F10)
8822-6850 eZKine™ Th1/Th17 Whole Blood Intracellular Cytokine Kit
8822-6851 eZKine™ Th1 Activation 1 Whole Blood Intracellular Cytokine Kit
8822-6852 eZKine™ Th1 Activation 2 Whole Blood Intracellular Cytokine Kit
8822-6853 eZKine™ Th17/Th22 Whole Blood Intracellular Cytokine Kit
8822-6854 eZKine™ CD8 Activation 1 Whole Blood Intracellular Cytokine Kit
8822-6855 eZKine™ CD8 Activation 2 Whole Blood Intracellular Cytokine Kit

Tel: 888.999.1371 or 858.642.2058 • Fax: 858.642.2046 • thermofisher.com/ebioscience • info@ebioscience.com

invitrogen

eZKine™ 4-Color Compensation Kit

Protocol: eZKine™ 4-Color Compensation Protocol

This kit is intended for use in compensation setup in experiments using eZKine™ Whole Blood Intracellular Cytokine Kits.

Materials Provided

- Refer to the components section of the datasheet.

Other Materials Needed

- 12 x 75 mm round bottom test tubes
- Flow Cytometry staining Buffer (cat. 00-4222)

Time Requirements

- 15-30 minute staining incubation
- 5 minute wash
- 5-20 minute instrument setup

Experimental Procedure

Preparation of Single-Color Compensation Controls

1. Label a tube for each of the 4 fluorochromes that will be used in the experiment.
2. Mix beads by vigorously inverting at least 10 times or pulse-vortexing.
3. Add 1 drop of OneComp eBeads to each tube.
4. Add 1 test or less of antibody conjugate to each tube.
   - A test is defined as the amount (µg) of antibody that will stain a cell sample in a final volume of 100 µL. If high background is observed on the negative bead population, less antibody can be used. For these cases, it is recommended to use 0.125 µg or less. Because the binding of the antibody to the positive bead is not dependent on the antibody's specificity, it is not necessary to use the antibody at its optimal concentration. For most antibodies, appropriate compensation values will result when 0.03-1.0 µg of antibody is used in a test.
5. Mix briefly by flicking or pulse-vortexing.
6. Incubate at 2-8°C for 15-30 minutes in the dark.
7. Add 2 mL of Flow Cytometry Staining Buffer to each tube and centrifuge at 400-600 x *g* for 3-5 minutes.
8. Decant supernatant and add 0.2-0.4 mL of Flow Cytometry Staining Buffer to each tube.
9. Mix briefly by flicking or pulse-vortexing before analysis.

General Compensation Setup Principles

10. Run unstained cells on cytometer. Determine appropriate FSC/SSC settings and fluorescence detector (PMT) voltages for the cells.
11. Run a sample of beads to adjust FSC/SSC to visualize beads (this can even be a single stained bead). It is OK to adjust the FSC/SSC to get the beads in view.
12. Run each single-stained bead sample to assure the positive peaks are on scale. PMT voltages should be decreased (as minimally as possible) for any positive bead peak that is off-scale. Do not record any data until all single-stained beads have been reviewed.
13. Run each single-stained bead sample to perform compensation setup and record files for compensation controls. For compensation setup, it is recommended to set a FSC/SSC gate around the major singlet population and use this for further fluorescence analysis.
14. Readjust FSC/SSC setting for cell samples, but do not adjust settings for fluorescence detectors.
15. Collect and record experimental samples.

Documentation and support

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Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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