

CD8 (RIV11) Mouse mAb

✓ 400 µl
(100 Tests)

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New 12/06

This product is for *in vitro* research use only and is not intended for use in humans or animals.

Applications	Species Cross-Reactivity	Molecular Wt.	Source	Isotype
F	H	32 kDa	Mouse	IgG1

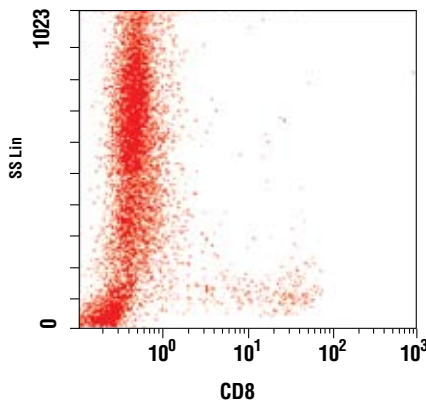
Background: Cluster of Differentiation 8 (CD8) is a disulphide-linked heterodimer consisting of the unrelated α and β subunits. Each subunit is a glycoprotein composed of a single extracellular Ig-like domain, a polypeptide linker, a transmembrane part and a short cytoplasmic tail. On T cells, CD8 is the coreceptor for the T cell receptor (TCR), and these two distinct structures recognize the Antigen-Major Histocompatibility Complex (MHC). Specifically, the Ig-like domain of CD8 α interacts with the $\alpha 3$ -domain of the MHC class I molecule. CD8 ensures specificity of the TCR-antigen interaction, prolongs the contact between the T cell and the antigen presenting cell, and the α chain recruits the tyrosine kinase Lck, which is essential for T cell activation (1).

Specificity/Sensitivity: CD8 (RIV11) Mouse mAb detects endogenous levels of total CD8 protein.

Source/Purification: Monoclonal antibody is produced by immunizing BALB/c mice with human peripheral lymphocytes.

Background References:

(1) Zamoyska, R. (1994) *Immunity* 1, 243-246.



Flow cytometric analysis of whole blood, using CD8 (RIV11) Mouse mAb.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at -20°C. Do not aliquot the antibody.

Recommended Antibody Dilutions:
Flow Cytometry 1:25

Companion Products:

CD10 (CB-CALLA) Mouse mAb #3565

CD13 (B-F10) Mouse mAb #3566

CD16 (FcγII) (CB-16) Mouse mAb #3567

CD34 (IC0115) Mouse mAb #3569

CD56 (NCAM) (123C3) Mouse mAb #3576

Flow Cytometry Protocol for Intracellular Staining Using Conjugated Secondary Antibodies

A Solutions and Reagents

1. **1X Phosphate Buffered Saline (PBS):** Dissolve 8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4 and 0.24 g KH_2PO_4 in 800 mL distilled water (dH_2O). Adjust the pH to 7.4 with HCl and the volume to 1 liter. Store at room temperature.
2. Formaldehyde (methanol free)
3. **Incubation Buffer:** Dissolve 0.5 g bovine serum albumin (BSA) in 100mL 1X PBS. Store at 4°C

B Fixation

1. Collect cells by centrifugation and aspirate supernatant.
2. Resuspend cells briefly in 0.5-1 ml PBS. Add formaldehyde to a final concentration of 2-4% formaldehyde.
3. Fix for 10 minutes at 37°C.
4. Chill tubes on ice for 1 minute.

C Permeabilization

1. Permeabilize cells by adding ice-cold 100% methanol slowly to pre-chilled cells, while gently vortexing, to a final concentration of 90% methanol. Alternatively, to remove fix prior to permeabilization, pellet cells by centrifugation and resuspend in 90% methanol.
2. Incubate 30 minutes on ice.
3. Proceed with staining or store cells at -20°C in 90% methanol.

D Staining Using Unlabeled Primary and Conjugated Secondary Antibodies

NOTE: Allow for isotype matched controls for monoclonal antibodies or species matched IgG for polyclonal antibodies. Count cells using a hemacytometer or alternative method.

1. Aliquot 0.5-1x10⁶ cells into each assay tube (by volume).
2. Add 2-3 ml Incubation Buffer to each tube and rinse by centrifugation. Repeat.
3. Resuspend cells in 100 μl Incubation Buffer per assay tube.
4. Block in Incubation Buffer for 10 minutes at room temperature.
5. Add the primary antibody at the appropriate dilution to the assay tubes (see individual antibody data sheet for the appropriate dilution).
6. Incubate for 30-60 minutes at room temperature.
7. Rinse as before in Incubation Buffer by centrifugation.
8. Resuspend cells in fluorochrome-conjugated secondary antibody*, diluted in Incubation Buffer according to the manufacturer's recommendations.
9. Incubate for 30 minutes at room temperature.
10. Rinse as before in Incubation Buffer by centrifugation.
11. Resuspend cells in 0.5 ml PBS and analyze on flow cytometer.

*Recommended Secondary Antibodies from Invitrogen.

A-11070 Alexa Fluor® 488 F(ab')₂ fragment of goat anti-rabbit IgG (H+L) (1:1000 dilution)
A-11017 Alexa Fluor® 488 F(ab')₂ fragment of goat anti-mouse IgG (H+L) (1:1000 dilution)