

Rabbit (DA1E) mAb IgG Isotype Control (Alexa Fluor® 647 Conjugate)

100 µl

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New 08/08

This product is for *in vitro* research use only and is not intended for use in humans or animals.
This product is not intended for use as a therapeutic or in diagnostic procedures.

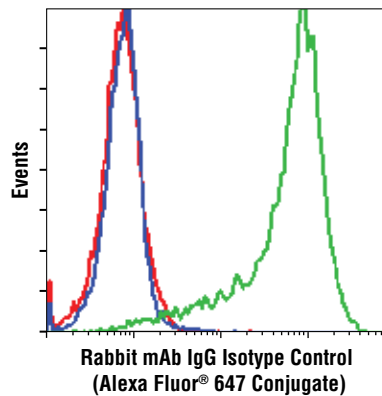
Concentration	Applications	Isotype
100 µg/ml	F	Rabbit IgG

Background: Isotype control antibodies are used to estimate the non-specific binding of target primary antibodies due to F_c receptor binding or other protein-protein interactions. An isotype control antibody should have the same immunoglobulin type as the test antibody.

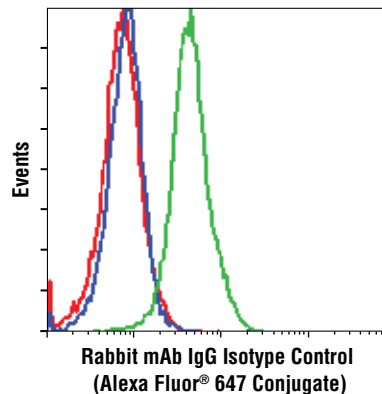
Description: Rabbit IgG was conjugated to Alexa Fluor® 647 fluorescent dye under optimal conditions, and tested in-house for direct flow cytometric analysis of human cells.

Specificity/Sensitivity: Normal Rabbit (DA1E) mAb IgG is not expected to recognize any specific antigen. It functions as an isotype control for rabbit monoclonal antibodies.

Directions for Use: Important! This control antibody must be diluted to the same concentration (not dilution) as the specific antibody in analysis. Higher background fluorescence may result if excessive amounts of rabbit IgG isotype control are used. Do not use this antibody at 1:10 dilution in the same way as recommended for other CST Alexa Fluor® conjugated antibodies.



Flow cytometric analysis of Jurkat cells, using Rabbit IgG Isotype Control (Alexa Fluor® 647 Conjugate) (red) compared to Phospho-S6 Ribosomal Protein (Ser235/236) (D57.2.2E) Rabbit mAb (Alexa Fluor® 647 Conjugate) #4851, untreated (blue) or IFN- α -treated (green).



Flow cytometric analysis of Jurkat cells, using Rabbit IgG Isotype Control (Alexa Fluor® 647 Conjugate) (red) compared to Phospho-Akt (Ser473) (D9E) Rabbit mAb (Alexa Fluor® 647 Conjugate) #4075, untreated (green) or LY294002- and wortmannin-treated (blue).

Storage: Supplied in PBS (pH 7.2), less than 0.1% sodium azide, 2 mg/ml BSA. Store at 4°C. *Protect from light. Do not freeze.*

Note: This control antibody must be diluted to the same concentration (not dilution) as the specific antibody in analysis. See Directions on Use.

Recommended Antibody Dilutions:

Flow Cytometry:
Match IgG concentration of primary antibody.

Companion Products:

Normal Rabbit (DA1E) mAb IgG #3900
Rabbit IgG Isotype Control (Alexa Fluor® 647 Conjugate) #3452
Phospho-Akt (Ser473) (D9E) Rabbit mAb (Alexa Fluor® 647 Conjugate) #4075
Phospho-S6 Ribosomal Protein (Ser235/236) (D57.2.2E) Rabbit mAb (Alexa Fluor® 647 Conjugate) #4851
Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (D13.14.4E) Rabbit mAb (Alexa Fluor® 647 Conjugate) #4284
Phospho-Akt (Ser473) (193H12) Rabbit mAb (Alexa Fluor® 647 Conjugate) #2337
Phospho-Stat3 (Tyr705) (D3A7) Rabbit mAb (Alexa Fluor® 647 Conjugate) #4324
Rabbit (DA1E) mAb IgG Isotype Control (Alexa Fluor® 488 Conjugate) #2975

The Alexa Fluor® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc., for research use only, except for use in combination with DNA microarrays. The Alexa Fluor® dyes (except for Alexa Fluor® 430 dye) are covered by pending and issued patents.

Alexa Fluor® is a registered trademark of Molecular Probes, Inc.

Flow Cytometry Protocol for Intracellular Staining Using Conjugated Primary 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 Conjugated Primary 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 5×10^5 cells into each assay tube (by volume).
2. Add 2-3 ml Incubation Buffer to each tube and rinse by centrifugation.
3. Resuspend cells in 90 μl Incubation Buffer per assay tube.
4. Block in Incubation Buffer for 10 minutes at room temperature.
5. Add 10 μl of conjugated antibody to the assay tubes.
6. Incubate for 30-60 minutes, in the dark at room temperature.
7. Rinse as before in Incubation Buffer by centrifugation.
8. Resuspend cells in 0.5 ml PBS and analyze on flow cytometer.