SONY

Product Data Sheet

Alexa Fluor® 488 anti-NFATc1

Catalog # / Size: 3848015 / 25 µg
3848020 / 100 µg

Clone: 7A6

Isotype: Mouse IgG1

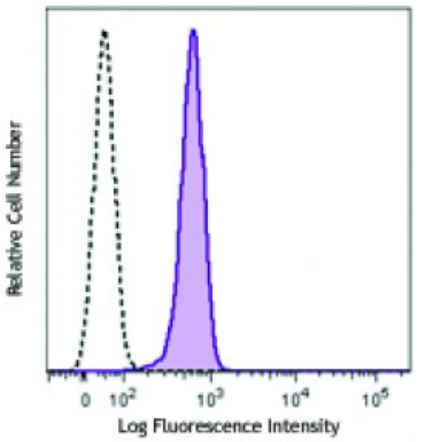
Immunogen: Recombinant protein of human NFATc1 amino acids 197-304.

Reactivity: Human,Mouse,Rat

Preparation: The antibody was purified by affinity chromatography and conjugated with Alexa Fluor® 488 under optimal conditions.

Formulation: Phosphate-buffered solution, pH 7.2, containing 0.09% sodium azide.

Concentration: 0.5



Human peripheral blood lymphocytes were treated with pre-chilled 1% PFA plus 70% ethanol, then intracellularly stained with NFATc1 (clone 7A6) Alexa Fluor® 488 (filled histogram) or mouse IgG1, κ Alexa Fluor® 488 isotype control (open histog

Applications:

Applications: Immunofluorescence

Recommended Usage: Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis. For flow cytometric staining, the suggested use of this reagent is ≤0.25 microg per million cells in 100 microL volume. It is recommended that the reagent be titrated for optimal performance for each application.

* Alexa Fluor® 488 has a maximum emission of 519 nm when it is excited at 488 nm.

Application Notes: 7A6 antibody detects endogenous human NFATC1 in Western blot with an approximate molecular weight of 100 - 120 kD (different isoforms) (Cat. No. 649602). The optimal concentration should be determined by titration for each individual assay of interest.

Clone 7A6 Intracellular Staining Procedure:

1. Prepare the fix/perm solution, consisting of 1% paraformaldehyde in 70% ethanol. Chill for at least one hour at -20°C.
2. Suspend cells in cell staining buffer and perform surface stain if necessary.
3. Centrifuge for 5 minutes at 1500 RPM and discard supernatant.
4. Resuspend cells in 500 µL of fix/perm solution for up to 2 x 10^6 cells and gently vortex.
5. Incubate cells on ice for 30-60 minutes in the dark.

6. Add 2 mL PBS and centrifuge cells for 1500 RPM for 5 minutes.

7. Wash cells 1X with 2 mL cell staining buffer, centrifuge at 1500 RPM for 5 minutes, discard supernatant.

8. Adjust cell concentration to about 10 x 10^7 cells/mL, add 100 µL (~ 1 x 10^6 cells) to a FACS tube that already contains the staining antibody and mix gently.

9. Incubate 15 minutes at room temperature.

10. Wash 2X with cell staining buffer as in step 6.

11. Resuspend cells in cell staining buffer and analyze.

Application References:

1. Timmerman LA, *et al.* 1997. *J. Immunol.* 159:2735. (IF)
2. Brandt C, *et al.* 2010. Cytometry A. 77:607. (FC)

Description:

The product of this gene is a component of the nuclear factor of activated T cells DNA-binding transcription complex. The protein complex consists of NFAT1, NFAT2 (NFATc1 or NFATc), NFAT3, and NFAT4. All members of this family are transcription factors with a Rel homology domain and regulate gene transcription in concert with AP-1 (Jun/Fos) to orchestrate an effective immune response. NFAT proteins are predominantly expressed in cells of the immune system but are also expressed in skeletal muscle, keratinocytes and adipocytes, regulating cell differentiation programs in these cells. In resting cells, NFAT proteins are heavily phosphorylated and localized in the cytoplasm. Increased intracellular calcium concentrations activate the calcium/calmodulin-dependent serine phosphatase calcineurin, which dephosphorylates NFAT proteins, resulting in their subsequent translocation to the nucleus.

Proteins belonging to this family of transcription factors play a central role in inducible gene transcription during immune response. The product of this gene is an inducible nuclear component. It functions as a major molecular target for the immunosuppressive drugs such as cyclosporin A. Five transcript variants encoding distinct isoforms have been identified for this gene. Different isoforms of this protein may regulate inducible expression of different cytokine genes.

Antigen References:

1. Zhao Q, *et al.* 2010. *Int. J. Biochem. Cell Biol.* 42:576.
2. Hoey T, *et al.* 1995. *Immunity* 2:461.
3. Northrop JP, *et al.* 1993. *J. Biol. Chem.* 268:2917.
4. Hogan PG, *et al.*