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Polyclonal Antibody to NFAT1 (NFATc2) pSer54 - Aff - Purified

Catalog No.: BP7135

Quantity: 0.1 ml

Background: Nuclear factor of activated T cells (NFAT) is a family of transcription factors implicated in multiple biological processes including cytokine gene expression, cardiac hypertrophy and adipocyte differentiation. NFAT1 (also known as NFATc2 or NFATp) is a 154 kDa member of this family that is regulated by the calcium-dependent phosphatase calcineurin. When calcineurin is activated by calcium it dephosphorylates multiple residues in the regulatory domain of NFAT1, leading to its translocation to the nucleus and activation of its transcriptional activity. Once in the nucleus, NFAT proteins act synergistically with the AP-1 transcription factor complex to regulate the expression of multiple genes. Serine 54 in mouse NFAT1 has been shown to be important in the regulation of its transcriptional activity.

Host: Rabbit

Immunogen: The antiserum was produced against a chemically synthesized phosphopeptide derived from a region of mouse NFAT1 that contains serine 54.

Format:
State: Liquid Ig fraction
Purification: Prepared from serum by sequential epitope-specific chromatography. The antibody has been negatively preadsorbed using a non-phosphopeptide corresponding to the site of phosphorylation to remove antibody that is reactive with non-phosphorylated NFAT1. The final product is generated by affinity chromatography using a NFAT1-derived peptide phosphorylated at serine 54.
Buffer System: Dulbeccos phosphate buffered saline (without Mg2+ and Ca2+), pH 7.3 (+/- 0.1), with 1.0 mg/mL BSA (IgG, protease free) as a carrier, containing 0.05% sodium azide

Applications: Western blotting: use at a 1:1000 starting dilution.
Positive control: Murine T-cells + PMA + Ionomycin
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: The antibody recognizes mouse NFAT1. Other species have not been tested.

Storage: Can be shipped at 2-8°C. Upon arrival, centrifuge before opening to settle vial contents. Then aliquot and store at -20°C. Avoid repeated freezing and thawing.
Shelf life: one year from despatch.

General Readings: Holmberg, C., et al. (2002) Multisite phosphorylation provides sophisticated regulation of transcription factors. Trends Biochem. Sci. 27(12):619-627.
Feske, S., et al. (2000) Impaired NFAT regulation and its role in a severe combined immunodeficiency. Immunobiology. 202(2):134-150.

Okamura, H., et al. (2000) Concerted dephosphorylation of the transcription factor NFAT1 induces a conformational switch that regulates transcriptional activity. Mol. Cell. 6(3):539-550.
Martelli, M.P., et al. (2000) Signaling via LAT (linker for T-cell activation) and Syk/ZAP70 is required for ERK activation and NFAT transcriptional activation following CD2 stimulation. Blood 96(6):2181-2190.
Fang, N., et al. (1996) Tyrosines 113, 128, and 145 of SLP-76 are required for optimal augmentation of NFAT promoter activity. J. Immunol. 157(9):3769-3773.

Protocols:

Western Blotting Procedure
1. Lyse approximately 10e7 cells in 0.5 mL of ice cold Cell Lysis Buffer (formulation provided below). This buffer, a modified RIPA buffer, is suitable for recovery of most proteins, including membrane receptors, cytoskeletal-associated proteins, and soluble proteins. Other cell lysis buffer formulations, such as Laemmli sample buffer and Triton-X 100 buffer, are also compatible with this procedure. Additional optimization of the cell stimulation protocol and cell lysis procedure may be required for each specific application.
2. Remove the cellular debris by centrifuging the lysates at 14,000 x g for 10 minutes. Alternatively, lysates may be ultracentrifuged at 100,000 x g for 30 minutes for greater clarification.
3. Carefully decant the clarified cell lysates into clean tubes and determine the protein concentration using a suitable method, such as the Bradford assay. Polypropylene tubes are recommended for storing cell lysates.
4. React an aliquot of the lysate with an equal volume of 2x Laemmli Sample Buffer (125 mM Tris, pH 6.8, 10% glycerol, 10% SDS, 0.006% bromophenol blue, and 130 mM dithiothreitol [DTT]) and boil the mixture for 90 seconds at 100°C.
5. Load 10-30 µg of the cell lysate into the wells of an appropriate single percentage or gradient minigel and resolve the proteins by SDS-PAGE.
6. In preparation for the Western transfer, cut a piece of PVDF membrane slightly larger than the gel. Soak the membrane in methanol for 1 minute, then rinse with ddH2O for 5 minutes.
7. Soak the membrane, 2 pieces of Whatman paper, and Western apparatus sponges in transfer buffer (formulation provided below) for 2 minutes.
8. Assemble the gel and membrane into the sandwich apparatus.
9. Transfer the proteins at 140 mA for 60-90 minutes at room temperature.
10. Following the transfer, rinse the membrane with Tris buffered saline for 2 minutes.
11. Block the membrane with blocking buffer (formulation provided below) overnight at 4oC.
12.
Incubate the blocked blot with primary antibody at a 1:1000 starting dilution in Tris buffered saline supplemented with 3% BSA and 0.1% Tween 20 for 2 hours at room temperature.
13. Wash the blot with several changes of Tris buffered saline supplemented with 0.1% Tween 20.
14. Detect the antibody band using an appropriate secondary antibody, such as goat F(ab)2 anti-rabbit IgG alkaline phosphatase conjugate or goat F(ab)2 anti-rabbit IgG horseradish peroxidase conjugate in conjunction with your chemiluminescence reagents and instrumentation.

Cell Lysis Buffer Formulation:
10 mM Tris, pH 7.4
100 mM NaCl
1 mM EDTA
1 mM EGTA

1 mM NaF
20 mM Na4P2O7
2 mM Na3VO4
0.1% SDS
0.5% sodium deoxycholate
1% Triton-X 100
10% glycerol
1 mM PMSF (made from a 0.3 M stock in DMSO)
or 1 mM AEBSF (water soluble version of PMSF)
60 µg/mL aprotinin
10 µg/mL leupeptin
1 µg/mL pepstatin
(alternatively, protease inhibitor cocktail such as Sigma catalog number P2714 may be used)

Transfer Buffer Formulation:
2.4 gm Tris base, 14.2 gm glycine, 200 mL methanol
Q.S. to 1 liter, then add 1 mL 10% SDS.
Cool to 4oC prior to use.

Tris Buffered Saline Formulation:
20 mM Tris-HCl, pH 7.4
0.9% NaCl

Blocking Buffer Formulation:
100 mL Tris buffered saline
5 gm BSA
0.1 mL Tween 20

Pictures:

Peptide Competition: Murine T cells were left untreated (1) or treated with PMA and Ca2+ ionophore ionomycin (2-5), and cell lysates were resolved by SDS-PAGE on a 10% polyacrylamide gel and transferred to PVDF. Membranes were blocked with a 5% BSA-TBST buffer overnight at 4°C, then were incubated with 0.50 µg/mL NFAT1 [pS54] (mouse) antibody for two hours at room temperature in a 3% BSA-TBST buffer, following prior incubation with: no peptide (1, 2), the non-phosphopeptide corresponding to the immunogen (3), a generic phosphoserine-containing peptide (4), or, the phosphopeptide immunogen (5). After washing, membranes were incubated with goat F(ab)2 anti-rabbit IgG alkaline phosphatase and signals were detected using the Tropix WesternStar(TM) method. The data show that only the peptide corresponding to NFAT1 [pS54] (mouse) blocks the antibody signal, thereby demonstrating the specificity of the antibody, and stimulation-induced serine phosphorylation of NFAT1.