Product Information

Anti-phospho-NFAT1 (pSer^{54})

Produced in rabbit, affinity isolated antibody

N8536

Product Description

Anti-phospho-NFAT1 (Nuclear Factor of Activated T cells) (pSer^{54}) is developed in rabbit using as immunogen a synthetic phosphorylated peptide derived from a region of mouse NFAT1 (Gene ID No. 18019) that contains Ser^{54}. The antiserum is affinity purified using epitope-specific affinity chromatography. The antibody is preadsorbed to remove any reactivity toward a non-phosphorylated peptide.

The antibody detects mouse NFAT1. Other species have not been tested. It has been used in immunoblotting applications.

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.

Ser^{54} in mouse NFAT1 has been shown to be important in the regulation of its transcriptional activity.

Reagent

Supplied as a solution in Dulbecco's phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.3, with 1.0 mg/mL BSA (IgG and protease free) and 0.05% sodium azide.

Precautions and Disclaimer

This product is for research use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store at –20 °C. Upon initial thawing freeze the solution in working aliquots for extended storage. Avoid repeated freezing and thawing to prevent denaturing the antibody. Do not store in frost-free freezers. Working dilution samples should be discarded if not used within 12 hours. The antibody is stable for at least 12 months when stored appropriately.

Product Profile

The supplied reagent is sufficient for 10 blots.

Immunoblotting: 1:1000.

Note: In order to obtain the best results in various techniques and preparations, we recommend determining optimal working concentration by titration.

Results

Peptide Competition

1. Murine T cells were left untreated (Lane 1) or treated with PMA and Ca^{2+} ionophore ionomycin (Lanes 2-5), and cell lysates were resolved by SDS-PAGE on a 10% polyacrylamide gel and transferred to PVDF.
2. Membranes were blocked with a 5% BSA-TBST buffer overnight at 4 °C.
3. After blocking, membranes were preincubated with different peptides as follows:

Lane 1, 2	no peptide
Lane 3	non-phosphorylated peptide corresponding to the immunogen
Lane 4	a generic phosphoserine containing peptide
Lane 5	immunogen

4. All lanes were incubated with 0.50 µg/mL NFAT1 ($pSer^{54}$) antibody for two hours at room temperature in a 3% BSA-TBST buffer.
5. After washing, membranes were incubated with goat $F(ab')_2$ anti-rabbit IgG alkaline phosphatase and signals were detected.

The data in Figure 1 show that only the peptide corresponding to NFAT1 ($pSer^{54}$) (mouse) blocks the antibody signal, thereby demonstrating the specificity of the antibody and stimulation-induced serine phosphorylation of NFAT1.

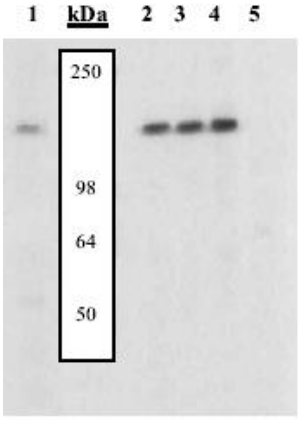

Figure 1 Peptide Competition

References

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