

# NFAT4 Antibody

✓ 100 µl  
(10 Western mini-blots)



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TECHNOLOGY®

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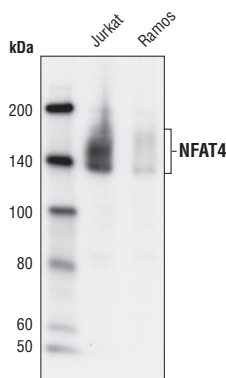
This product is for *in vitro* research use only and is not intended for use in humans or animals.

Applications W, IP	Species Cross-Reactivity H (M)	Molecular Wt. 140–190 kDa	Source Rabbit
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**Background:** The NFAT (nuclear factor of activated T cells) family of proteins consists of NFAT1 (NFATc2 or NFATp), NFAT2 (NFATc1 or NFATc), NFAT3 (NFATc4) and NFAT4 (NFATc3 or NFATx). 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 (1,2). 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 (3). In resting cells, NFAT proteins are heavily phosphorylated and localized in the cytoplasm. Increased intracellular Ca<sup>2+</sup> concentrations activate the Ca<sup>2+</sup>/calmodulin-dependent serine phosphatase calcineurin, which dephosphorylates NFAT proteins, which subsequently translocate to the nucleus (2). Termination of NFAT signaling occurs upon declining Ca<sup>2+</sup> concentrations and rephosphorylation of NFAT by kinases such as GSK-3 or CK1 (3,4). Cyclosporine A and FK506 are immunosuppressive drugs that inhibit calcineurin and thus restrain NFAT proteins in the cytoplasm (5). NFAT4 upregulates the expression of several cytokine genes and activation markers and downregulates expression of CD154 in naive CD4(+) T cells. Furthermore, NFAT4 augments expression of IFN-γ and TNF-α in helper T-cells 1 (Th1 cells) and attenuates expression of IL-4 and IL-5 in helper T-cells 2 (Th2 cells) (6).

**Specificity/Sensitivity:** NFAT4 Antibody detects endogenous levels of total NFAT4 protein.

**Source/Purification:** Polyclonal antibodies are produced by immunizing rabbits with a synthetic peptide (KLH-coupled) derived from the sequence of human NFAT4. Antibodies are purified by protein A and peptide affinity chromatography.



Western blot analysis of extracts from Jurkat and Ramos cells, using NFAT4 Antibody.

**Background References:**

- (1) Northrop, J.P. et al. (1993) *J. Biol. Chem.* 268, 2917–2923.
- (2) Hogan, P.G. et al. (2003) *Genes Dev.* 17, 2205–2232.
- (3) Crabtree, G.R. and Olson, E.N. (2002) *Cell* 109, S67–S79.
- (4) Okamura, H. et al. (2004) *Mol. Cell. Biol.* 24, 4184–4195.
- (5) Shaw, K.T. et al. (1995) *Proc. Natl. Acad. Sci. USA* 92, 11205–11209.
- (6) Chen, J. et al. (2003) *J. Immunol.* 170, 3109–3117.

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

**Recommended Antibody Dilutions:**

Western blotting 1:1000

**Companion Products:**

- SignalSilence™ GSK-3α/β siRNA Kit #6300
- SignalSilence™ GSK-3α siRNA #6312
- Phospho-GSK-3α/β (Ser21/9) Antibody #9331
- GSK-3β (27C10) Rabbit mAb #9315
- Phospho-GSK-3β (Ser9) Antibody #9336
- Phospho-GSK-3α (Ser21) (46H12) Mouse mAb #9337
- GSK-3α Antibody #9338
- HTScan™ GSK3-β Kinase #7545
- Anti-rabbit IgG, HRP-linked Antibody #7071
- Anti-rabbit IgG, HRP-linked Antibody #7074
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- LumiGLO® Reagent and Peroxide #7003

**IMPORTANT: For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.**

## Western Immunoblotting Protocol

**For Western blots, incubate membrane with diluted anti-body in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.**

### A. Solutions and Reagents

Note: Prepare solutions with Milli-Q or equivalently purified water.

- A1. ■ 1X Phosphate Buffered Saline (PBS)
- A2. ■ 1X SDS Sample Buffer:  
62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
- A3. ■ Transfer Buffer:  
25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- A4. ■ 10X Tris Buffered Saline (TBS):  
To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
- A5. ■ Nonfat Dry Milk (weight to volume [w/v])
- A6. ■ Blocking Buffer:  
1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
- A7. ■ Wash Buffer:  
1X TBS, 0.1% Tween-20 (TBS/T)
- A8. ■ Bovine Serum Albumin (BSA)
- A9. ■ Primary Antibody Dilution Buffer:  
1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
- A10. ■ Phototope<sup>®</sup>-HRP Western Blot Detection System #7071:  
Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO<sup>®</sup> chemiluminescent reagent and peroxide.
- A11. ■ Prestained Protein Marker, Broad Range (Premixed Format) #7720
- A12. ■ Biotinylated Protein Ladder Detection Pack #7727
- A13. ■ Blotting Membrane:  
This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

### B. Protein Blotting

A general protocol for sample preparation is described below.

- B1. Treat cells by adding fresh media containing regulator for desired time.
- B2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- B3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
- B4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- B5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- B6. Microcentrifuge for 5 minutes.
- B7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

*Note: CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.*

- B8. Electrotransfer to nitrocellulose or PVDF membrane.

### C. Membrane Blocking and Antibody Incubations

*Note: Volumes are for 10 cm x 10 cm (100 cm<sup>2</sup>) of membrane; for different sized membranes, adjust volumes accordingly.*

- C1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- C2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- C3. Wash three times for 5 minutes each with 15 ml of TBS/T.
- C4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- C5. Wash three times for 5 minutes each with 15 ml of TBS/T.
- C6. Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
- C7. Wash three times for 5 minutes each with 15 ml of TBS/T.

### D. Detection of Proteins

- D1. Incubate membrane with 10 ml LumiGLO<sup>®</sup> (0.5 ml 20X LumiGLO<sup>®</sup>, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

*Note: LumiGLO<sup>®</sup> substrate can be further diluted if signal response is too fast.*

- D2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

*Note: Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO<sup>®</sup> incubation and declines over the following 2 hours.*

**A. Solutions and Reagents**

*Note: Prepare solutions with Milli-Q or equivalently purified water.*

- A1. ■ 1X Phosphate Buffered Saline (PBS)
- A2. ■ 1X Cell Lysis Buffer:  
20 mM Tris (pH 7.5)  
150 mM NaCl  
1 mM EDTA  
1 mM EGTA  
1% Triton X-100  
2.5 mM Sodium pyrophosphate  
1 mM  $\beta$ -Glycerolphosphate  
1 mM  $\text{Na}_3\text{VO}_4$   
1  $\mu\text{g}/\text{ml}$  Leupeptin
- Note: CST recommends adding 1 mM PMSF before use\*.*
- A3. ■ Transfer Buffer:  
25 mM Tris base, 0.2 mM glycine, 20% methanol (pH 8.5)
- A4. ■ Protein A Agarose Beads:  
(Can be stored for 2 weeks at 4°C.) Add 5 ml of 1X PBS to 1.5 g of protein A agarose beads. Shake 2 hours at 4°C; spin down. Wash pellet twice with PBS. Resuspend beads in 1 volume of PBS.
- A5. ■ 3X SDS Sample Buffer:  
187.5 mM Tris-HCl (pH 6.8 at 25°C), 6% w/v SDS, 30% glycerol, 150 mM DTT, 0.03% w/v bromophenol blue

**B. Preparing Cell Lysates**

- B1. Aspirate media. Treat cells by adding fresh media containing regulator for desired time.
- B2. To harvest cells under nondenaturing conditions, remove media and rinse cells once with ice-cold PBS.
- B3. Remove PBS and add 0.5 ml 1X ice-cold cell lysis buffer plus 1 mM PMSF\* to each plate (10 cm) and incubate the plate on ice for 5 minutes.
- B4. Scrape cells off the plate and transfer to microcentrifuge tubes. Keep on ice.
- B5. Sonicate on ice four times for 5 seconds each.
- B6. Microcentrifuge for 10 minutes at 4°C, and transfer the supernatant to a new tube. The supernatant is the cell lysate. If necessary, lysate can be stored at -80°C.

**C. Immunoprecipitation**

- C1. Take 200  $\mu\text{l}$  cell lysate and add primary antibody; incubate with gentle rocking overnight at 4°C.
- C2. Add protein A agarose beads (20  $\mu\text{l}$  of 50% bead slurry). Incubate with gentle rocking for 1–3 hours at 4°C.
- C3. Microcentrifuge for 30 seconds at 4°C. Wash pellet five times with 500  $\mu\text{l}$  of 1X cell lysis buffer. Keep on ice during washes.
- C4. Resuspend the pellet with 20  $\mu\text{l}$  3X SDS sample buffer. Vortex, then microcentrifuge for 30 seconds.
- C5. Heat the sample to 95–100°C for 2–5 minutes.
- C6. Load the sample (15–30  $\mu\text{l}$ ) on SDS-PAGE gel (12–15%).
- C7. Analyze sample by Western blotting (see Western Immunoblotting Protocol).