Phospho-ATF2 (Thr71) Polyclonal Antibody

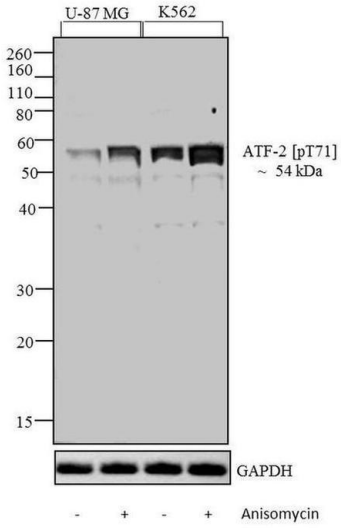
Product Details	
Size	100 µL
Species Reactivity	Human
Host/Isotype	Rabbit / IgG
Class	Polyclonal
Type	Antibody
Conjugate	Unconjugated
Immunogen	Chemically synthesized phosphopeptide derived from a region of human ATF2 that contains threonine 71
Form	Liquid
Purification	Antigen affinity chromatography
Storage buffer	Dulbecco's PBS, pH 7.3, with 1mg/mL BSA
Contains	0.05% sodium azide
Storage conditions	-20°C
RRID	AB_2533624

Applications	Tested Dilution	Publications
Western Blot (WB)	1:1,000	-
Immunohistochemistry (Paraffin) (IHC (P))	1:20	-

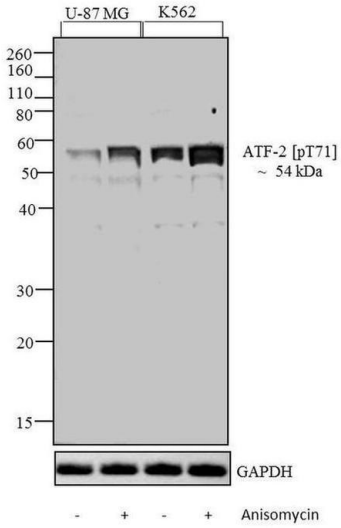
Product Specific Information

This antibody has been negatively preadsorbed using a non-phosphopeptide corresponding to the site of phosphorylation to remove antibody that is reactive with non phosphorylated ATF2. The final product is generated by affinity chromatography using an ATF2-derived peptide phosphorylated at threonine 71. The peptide sequence is conserved in mouse, rat, chicken and frog. Suggested Western blot positive controls: NIH3T3 + anisomycin, or Jurkat + 10 µg/mL anisomycin for 60 minutes.

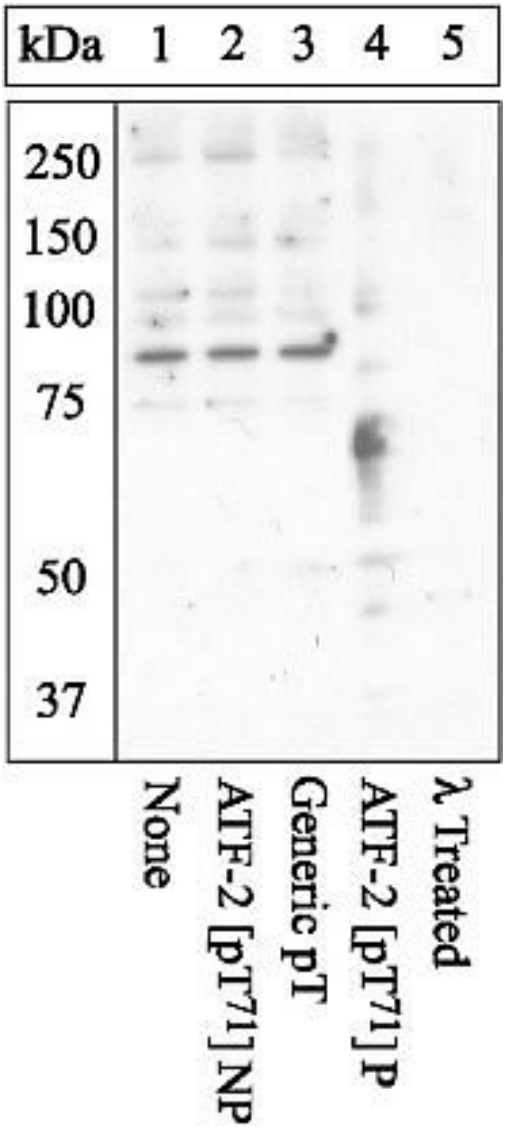
Product Images For Phospho-ATF2 (Thr71) Polyclonal Antibody



Phospho-ATF2 (Thr71) Antibody (44-295G)
Altered expression of proteins upon cell treatment demonstrates antibody specificity. Western blot using Phospho-ATF2 (Thr71) Polyclonal Antibody (Product # 44-295G), shows increased levels of phospho-ATF2 (Thr71) in U-87MG and K562 cell lines upon Anisomycin treatment. {TM}



Phospho-ATF2 (Thr71) Antibody (44-295G) in WB
Western blot analysis was performed on whole cell extracts (30 µg lysate) of U-87 MG (Lane 1), U-87 MG treated with 25 µg/mL anisomycin for 30 minutes (Lane 2), K562 (Lane 3), K562 treated with 25 µg/mL anisomycin for 30 minutes (Lane 4). The blots were probed with Anti-Phospho-ATF-2 pThr71 Rabbit Polyclonal Antibody (Product # 44-295G, 1-2 µg/mL) and detected by chemiluminescence using Goat anti-Rabbit IgG (H+L) Secondary Antibody, HRP conjugate (Product # G-21234, 1:5000 dilution). A ~ 54 kDa band corresponding to Phospho-ATF-2 pThr71 was observed across cell lines tested. Known quantity of protein samples were electrophoresed using Novex® NuPAGE® 10 % Bis-Tris gel (Product # NP0302BOX), XCell SureLock™ Electrophoresis System (Product # EI0002) and Novex® Sharp Pre-Stained Protein Standard (Product # LC5800). Resolved proteins were then transferred onto a nitrocellulose membrane with iBlot® 2 Dry Blotting System (Product # IB21001). The membrane was probed with the relevant primary and secondary Antibody following blocking with 5 % skimmed milk. Chemiluminescent detection was performed using Pierce™ ECL Western Blotting Substrate (Product # 32106).



Phospho-ATF2 (Thr71) Antibody (44-295G) in WB
Extracts of Jurkat cells treated with 10 µg/mL anisomycin for 60 minutes were resolved by SDS-PAGE on a 4-12% Bis-Tris gel and transferred to PVDF. The membrane was blocked with a 5% BSA-TBST buffer for one hour at room temperature and either left untreated (1-4) or treated with lambda () phosphatase (5), then incubated with the ATF2 (pT71) antibody for two hours at room temperature in a 3% BSA-TBST buffer, following prior incubation with: no peptide (1, 5), the phosphopeptide immunogen (2), the non-phosphopeptide corresponding to the phosphopeptide immunogen (3), or a generic phosphothreonine-containing peptide (4). After washing, the membrane was incubated with goat F (ab')2 anti rabbit IgG alkaline phosphatase (Product # ALI4405) and signals were detected using the Pierce SuperSignal™ method. The data show that only the phosphopeptide corresponding to ATF2 (pT71) blocks the antibody signal, demonstrating the specificity of the antibody. The data also show that phosphatase stripping eliminates the signal, further verifying that the antibody is phospho-specific.

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