Human/Mouse/Rat ATF6 Antibody

Recombinant Monoclonal Rabbit IgG Clone # 2358C
Catalog Number: MAB71527

DESCRIPTION

Species Reactivity	Human/Mouse/Rat
Specificity	Detects human ATF6 in direct ELISAs. Detects human, mouse, and rat ATF6 in Western blots.
Source	Recombinant Monoclonal Rabbit IgG Clone # 2358C
Purification	Protein A or G purified from cell culture supernatant
Immunogen	*E. coli*-derived recombinant human ATF6 Met1-Thr192 Accession # P18850
Formulation	Lyophilized from a 0.2 μm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 μm filtered solution in PBS.

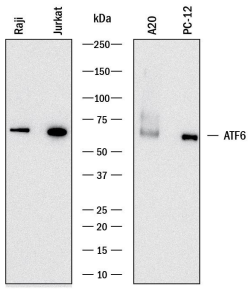
APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Western Blot	0.05 µg/mL	See Below
Immunocytochemistry	8-25 µg/mL	See Below
Immunohistochemistry	0.3-25 µg/mL	See Below
Intracellular Staining by Flow Cytometry	0.25 µg/10^6 cells	See Below

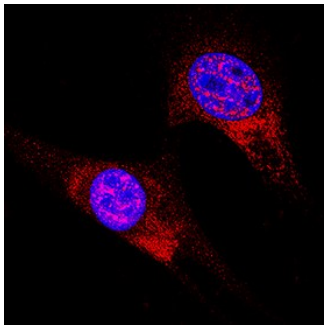
DATA

Western Blot



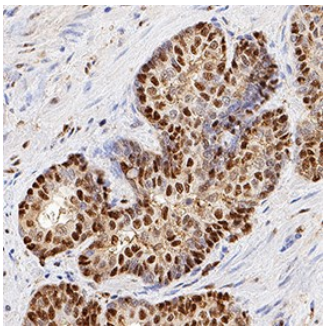
Detection of Human, Mouse, and Rat ATF6 by Western Blot. Western blot shows lysates of Raji human Burkitt's lymphoma cell line, Jurkat human acute T cell leukemia cell line, A20 mouse B cell lymphoma cell line, and PC-12 rat adrenal pheochromocytoma cell line. PVDF membrane was probed with 0.05 µg/mL of Rabbit Anti-Human/Mouse/Rat ATF6 Monoclonal Antibody (Catalog # MAB71527) followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). A specific band was detected for ATF6 at approximately 70 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunocytochemistry



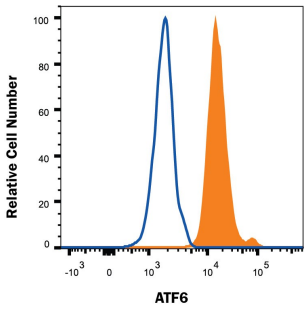
ATF6 in A549 Human Cell Line. ATF6 was detected in immersion fixed A549 human lung carcinoma cell line using Rabbit Anti-Human/Mouse/Rat ATF6 Monoclonal Antibody (Catalog # MAB71527) at 8 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Rabbit IgG Secondary Antibody (red; Catalog # NL004) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm and nuclei. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Immunohistochemistry



ATF6 in Human Prostate. ATF6 was detected in immersion fixed paraffin-embedded sections of human prostate using Rabbit Anti-Human/Mouse/Rat ATF6 Monoclonal Antibody (Catalog # MAB71527) at 0.3 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Rabbit IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC003). Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using Antigen Retrieval Reagent-Basic (Catalog # CTS013). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to cytoplasm and nuclei. View our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

Intracellular Staining by Flow Cytometry



Detection of ATF6 in Human HeLa cells by Flow Cytometry. HeLa human cervical epithelial cell line was stained with Rabbit Anti-Human/Mouse/Rat ATF6 Monoclonal Antibody (Catalog # MAB71527, filled histogram) or Rabbit IgG control antibody (Catalog # MAB1050, open histogram) followed by APC-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # F0111). To facilitate intracellular staining, cells were fixed and permeabilized with FlowX FoxP3 Fixation & Permeabilization Buffer Kit (Catalog # FC012). View our protocol for Staining Intracellular Molecules.

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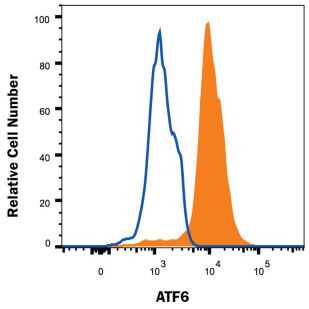
Intracellular Staining by Flow Cytometry



Detection of ATF6 in Human MCF-7 cells by Flow Cytometry. MCF-7 human breast cancer cell line was stained with Rabbit Anti-Human/Mouse/Rat ATF6 Monoclonal Antibody (Catalog # MAB71527, filled histogram) or Rabbit IgG control antibody (Catalog # MAB1050, open histogram) followed by APC-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # F0111). To facilitate intracellular staining, cells were fixed and permeabilized with FlowX FoxP3 Fixation & Permeabilization Buffer Kit (Catalog # FC012). View our protocol for Staining Intracellular Molecules.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 12 months from date of receipt, -20 to -70 °C as supplied. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

ATF6 is a constitutively expressed, endoplasmic reticulum (ER) membrane-anchored transcription factor. ATF6 is a key transcriptional activator of the unfolded protein response (UPR), which allows mammalian cells to maintain cellular homeostasis when they are subjected to environmental and physiological stresses that target the ER (reviewed in Shen, 2005 & Prywes, 2005). The C-terminus of ATF6 is located in the ER lumen and its N-terminal DNA binding domain faces the cytosol. AFT6 plays a key role in the ER stress response by transmitting the ER stress signal across the ER membrane into the nucleus. The induction of new gene expression by ATF6 is an important aspect of the ER stress response. In response to certain stress conditions, ATF6 translocates from the ER to the Golgi. The 90 kDa full-length ATF6 is processed within the Golgi to its active 50 kDa form through sequential cleavage by site-1 and site-2 proteases (S1P and S2P). Proteolytic activation of ATF6 in the ER stress response is a mechanism to regulate membrane-bound factors, and is referred to as regulated intramembrane proteolysis. The N-terminal active ATF6 translocates to the nucleus where it binds to ER stress-response elements in ER stress-response genes (ERSRGs). ATF6 is a potent transcriptional activator of ERSRGs. The fully glycosylated form of ATF6, a 670 amino acid protein, exhibits an electrophoretic mobility of ~90 kDa in denaturing SDS-gels, in part because of the glycosylated modifications. ATF6 has 3 consensus sites for N-linked glycosylation and exists constitutively as a glycosylated protein. Differentially glycosylated ATF6 forms may result from mutations or experimental treatment.

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