Human/Mouse/Rat AIF Antibody

Antigen Affinity-purified Polyclonal Rabbit IgG
Catalog Number: AF1457

DESCRIPTION

Species Reactivity	Human/Mouse/Rat
Specificity	Detects human, mouse, and rat mitochondria-processed AIF.
Source	Polyclonal Rabbit IgG
Purification	Antigen Affinity-purified
Immunogen	*E. coli*-derived recombinant human AIF Glu121-Asp613 Accession # O95831
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.

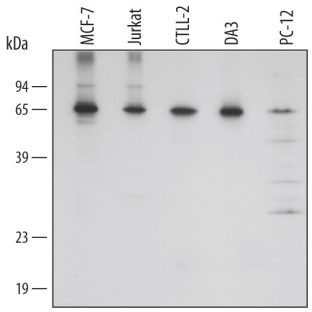
APPLICATIONS

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	0.1 µg/mL	See Below
Immunocytochemistry	5-15 µg/mL	See Below
Simple Western	1 µg/mL	See Below
Knockout Validated	AIF is specifically detected in HEK293T human embryonic kidney parental cell line but is not detectable in AIF knockout HEK293T cell line.	

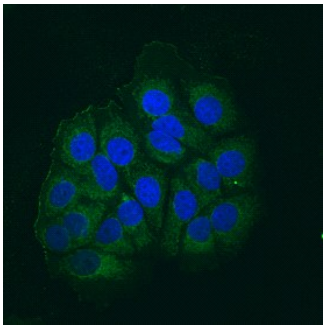
DATA

Western Blot



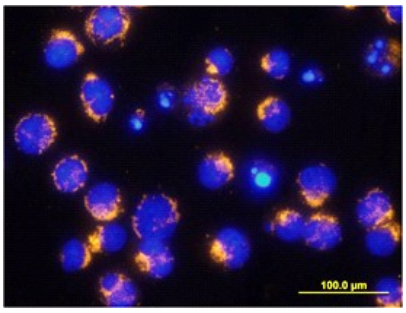
Detection of Human/Mouse/Rat AIF by Western Blot. Western blot shows lysates of MCF-7 human breast cancer cell line, Jurkat human acute T cell leukemia cell line, CTLL-2 mouse cytotoxic T cell line, DA3 mouse myeloma cell line, and PC-12 rat adrenal pheochromocytoma cell line. PVDF membrane was probed with 0.1 µg/mL of Rabbit Anti-Human/Mouse/Rat AIF Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1457) followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). A specific band was detected for AIF at approximately 67 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 2.

Immunocytochemistry



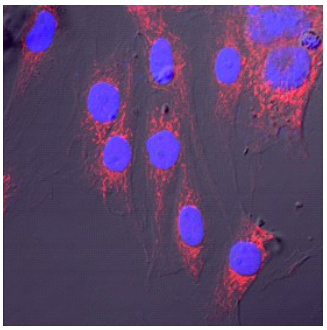
AIF in MCF-7 Human Cell Line. Apoptosis Inducing Factor (AIF) was detected in immersion fixed MCF-7 human breast cancer cell line using Rabbit Anti-Human/Mouse/Rat AIF Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1457) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 493-conjugated Anti-Rabbit IgG Secondary Antibody (green; Catalog # NL006) and counterstained with DAPI (blue). View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Immunocytochemistry



AIF in Jurkat Human Cell Line. AIF was detected in immersion fixed staurosporine-stimulated Jurkat human acute T cell leukemia cell line using Rabbit Anti-Human/Mouse/Rat AIF Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1457) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the Northern-Lights™ 557-conjugated Anti-Rabbit IgG Secondary Antibody (yellow; Catalog # NL004) and counterstained with DAPI (blue). View our protocol for Fluorescent ICC Staining of Non-adherent Cells.

Immunocytochemistry



AIF in HeLa Human Cell Line. AIF was detected in immersion fixed HeLa human cervical epithelial carcinoma cell line using Rabbit Anti-Human/Mouse/Rat AIF Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1457) at 15 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Sheep IgG Secondary Antibody (red; Catalog # NL010) and counterstained with DAPI (blue). Specific staining was localized to mitochondria. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

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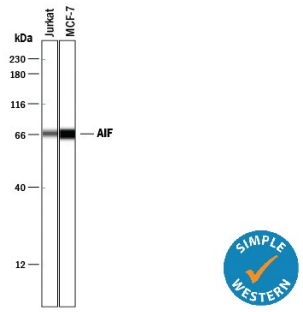
Human/Mouse/Rat AIF Antibody

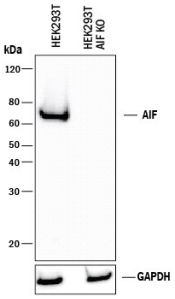
Antigen Affinity-purified Polyclonal Rabbit IgG
Catalog Number: AF1457

Simple Western



Detection of Human AIF by Simple Western™. Simple Western lane view shows lysates of Jurkat human acute T cell leukemia cell line and MCF-7 human breast cancer cell line, loaded at 0.2 mg/mL. A specific band was detected for AIF at approximately 69 kDa (as indicated) using 1 µg/mL of Rabbit Anti-Human/Mouse/Rat AIF Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1457). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

Knockout Validated



Western Blot Shows Human AIF Specificity by Using Knockout Cell Line. Western blot shows lysates of HEK293T human embryonic kidney parental cell line and AIF knockout HEK293T cell line (KO). PVDF membrane was probed with 0.1 µg/mL of Rabbit Anti-Human/Mouse/Rat AIF Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1457) followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). A specific band was detected for AIF at approximately 65 kDa (as indicated) in the parental HEK293T cell line, but is not detectable in knockout HEK293T cell line. GAPDH (Catalog # AF5718) is shown as a loading control. This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 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

Apoptosis-inducing factor (AIF, also known as programmed cell death protein 8) is a 58 kDa member of the FAD-dependent oxidoreductase family of molecules. It is ubiquitously expressed and found in the mitochondrial intermembrane space. AIF likely acts as a mitochondrial antioxidant providing protection via NADH oxidase activity. Upon release from the mitochondria, AIF passes into the nucleus where it initiates apoptosis. Human AIF precursor is 67 kDa in size and 613 amino acids (aa) in length and contains a cleavable N-terminal 102 aa mitochondrial localization sequence, followed by a spacer region (aa 103-129) and an oxidoreductase domain (aa 130-613) that possesses an NLS (aa 446-451). Over aa 121-613, human AIF shares 95% aa identity with mouse AIF.

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