Human/Mouse/Rat BAK Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF8161

DESCRIPTION

Species Reactivity	Human/Mouse/Rat
Specificity	Detects endogenous human, mouse and rat BAK in Western blots.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	*E. coli*-derived recombinant human BAK Pro20-Asn124 Accession # Q16611
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	1 µg/mL	See Below
Immunocytochemistry	5-15 µg/mL	See Below
Immunohistochemistry	5-15 µg/mL	Immersion fixed paraffin-embedded sections of Human Colon
Simple Western	4 µg/mL	Jurkat human acute T cell leukemia cell line and THP-1 human acute monocytic leukemia cell line

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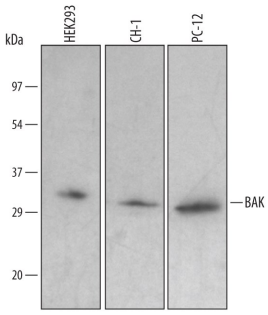
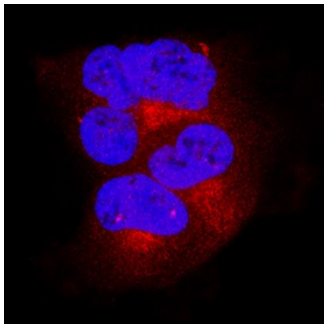
DATA

Western Blot



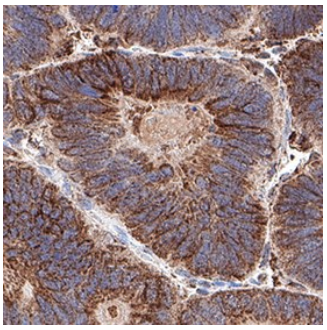
Detection of Human/Mouse/Rat BAK by Western Blot. Western blot shows lysates of HEK293 human embryonic kidney cell line, CH-1 mouse B cell lymphoma cell line, and PC-12 rat adrenal pheochromocytoma cell line. PVDF membrane was probed with 1 µg/mL of Human/Mouse/Rat BAK Antigen Affinity-purified Polyclonal Antibody (Catalog # AF8161) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF109). A specific band was detected for BAK at approximately 29 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 2.

Immunocytochemistry



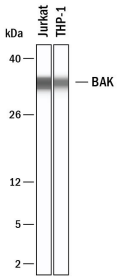
BAK in HEK293 Human Cell Line. BAK was detected in immersion fixed HEK293 human embryonic kidney cell line using Goat Anti-Human/Mouse/Rat BAK Antigen Affinity-purified Polyclonal Antibody (Catalog # AF8161) at 15 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Goat IgG Secondary Antibody (red; Catalog # NL001) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Immunohistochemistry



Detection of BAK in Human Colon. BAK was detected in immersion fixed paraffin-embedded sections of Human Colon using Goat Anti-Human/Mouse/Rat BAK Antigen Affinity-purified Polyclonal Antibody (Catalog # AF8161) at 5 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Goat IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC004). Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using VisUCyte Antigen Retrieval Reagent-Basic (Catalog # VCTS021). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to cell membrane in epithelial cells in mucosal glands. View our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

Simple Western



Detection of Human BAK by Simple Western™. Simple Western shows lysates of Jurkat human acute T cell leukemia cell line and THP-1 human acute monocytic leukemia cell line, loaded at 0.5 mg/ml. A specific band was detected for BAK at approximately 34 kDa (as indicated) using 4 µg/mL of Goat Anti-Human/Mouse/Rat BAK Antigen Affinity-purified Polyclonal Antibody (Catalog # AF8161). This experiment was conducted under reducing conditions and using the 2-40kDa separation system.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS. For liquid material, refer to CoA for concentration.
Shipping	Lyophilized product is shipped at ambient temperature. Liquid small pack size (-SP) is shipped with polar packs. Upon receipt, store immediately at the temperature recommended below.
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

BAK (Bcl-2 homologous antagonist/killer; also BAK1) is a 25-30 kDa member of the BCL-2 family of proteins. It is widely expressed, and participates in the apoptotic cycle. BAK is an outer mitochondrial membrane protein that is inactive as a Zn-dependent homodimer. Upon activation by p53 or tBID, BAK oligomerizes, creating a pore in the mitochondrial membrane and allowing for cytochrome C release. Human BAK is 211 amino acids (aa) in length and contains three BCL-2 homology domains (aa 74-88, 117 -136 and 169-184), a Zn-binding region (aa 160-166) and a C-terminal transmembrane segment (aa 188-205). Amino acids 67-94 mediate oligomerization of BAK. There are two potential isoform variants; one shows an alternate start site at Met96, while a second shows a deletion of aa 46-66. Over amino acids 20-124, human BAK shares 76% aa identity with mouse BAK.

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