Human Cytokeratin 18 Antibody

Antigen Affinity-purified Polyclonal Sheep IgG
Catalog Number: AF7619

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human Cytokeratin 18 in direct ELISAs and Western blots. In direct ELISAs, less than 1% cross-reactivity with recombinant human Cytokeratin 14 (KRT14) is observed.
Source	Polyclonal Sheep IgG
Purification	Antigen Affinity-purified
Immunogen	*E. coli*-derived recombinant human Cytokeratin 18 Ala239-Asp397 Accession # P05783
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.

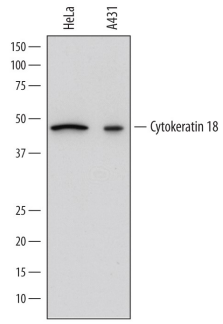
APPLICATIONS

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
Immunohistochemistry	3-15 µg/mL	See Below
Simple Western	1 µg/mL	See Below

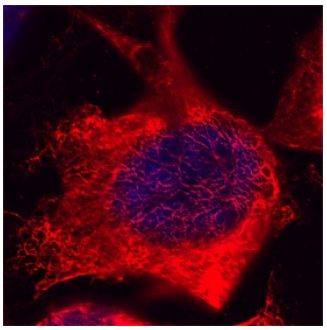
DATA

Western Blot



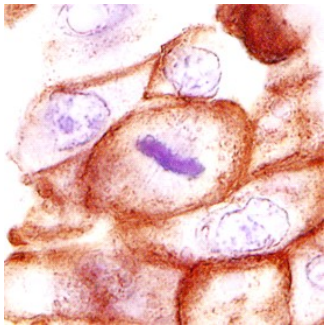
Detection of Human Cytokeratin 18 by Western Blot. Western blot shows lysates of HeLa human cervical epithelial carcinoma cell line and A431 human epithelial carcinoma cell line. PVDF membrane was probed with 0.1 µg/mL of Sheep Anti-Human Cytokeratin 18 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF7619) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). A specific band was detected for Cytokeratin 18 at approximately 46 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunocytochemistry



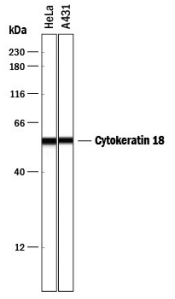
Cytokeratin 18 in HeLa Human Cell Line. Cytokeratin 18 was detected in immersion fixed HeLa human cervical epithelial carcinoma cell line using Sheep Anti-Human Cytokeratin 18 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF7619) at 5 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Sheep IgG Secondary Antibody (red; Catalog # NL010) and counterstained with DAPI (blue). Specific staining was localized to cytoskeletal fibers. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Immunohistochemistry



Cytokeratin 18 in Human Breast Cancer Tissue. Cytokeratin 18 was detected in immersion fixed paraffin-embedded sections of human breast cancer tissue using Sheep Anti-Human Cytokeratin 18 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF7619) at 3 µg/mL overnight at 4 °C. 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 the Anti-Sheep HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS019) and counterstained with hematoxylin (blue). Specific staining was localized to intermediate filaments. View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.

Simple Western



Detection of Human Cytokeratin 18 by Simple Western™. Simple Western lane view shows lysates of HeLa human cervical epithelial carcinoma cell line and A431 human epithelial carcinoma cell line, loaded at 0.2 mg/mL. A specific band was detected for Cytokeratin 18 at approximately 57 kDa (as indicated) using 1 µg/mL of Sheep Anti-Human Cytokeratin 18 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF7619) followed by 1:50 dilution of HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

PREPARATION AND STORAGE

Reconstitution	Sterile PBS to a final concentration of 0.2 mg/mL.
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.

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BACKGROUND

Cytokeratin 18; also KRT-18 (Keratin, type I cytoskeletal 18), Cell proliferation-inducing gene 46 and Keratin-18) is a 44-46 kDa Class I (large keratins of acidic pH) member of the intermediate filament family of proteins. Individual keratins are always expressed in tandem with a second keratin, and these are found in all epithelial cells. The class I Cytokeratin 18 heterodimerizes/polymerizes with 50-52 kDa class II KRT-8 to form 8-10 nm filaments in single strata plus hepatic epithelia. Cytokeratin 18 and -8 are the first keratins to appear in the mammalian embyro. In the adult, Cytokeratin 18 appears to participate in subtractions and additions to the plasma membrane. In this regard, a number of intracellular proteins interact with Cytokeratin 18, including 14-3-3, HSPc70 and Mrj. Cytokeratin 18 may also be O-glycosylated, and when so, serves to promote Akt-1 activity, thus protecting against apoptosis. Human Cytokeratin 18 is 430 amino acids (aa) in length. It contains an N-terminal "head" region (aa 1-79), a subsequent "rod" region (aa 80-387) with two coiled segments, and a C-terminal tail region. Cytokeratin 18 possesses at least 19 utilized phosphorylation sites plus five acetylated Lys residues. There are multiple isoforms that range from 20-40 kDa in size and are the result of caspase cleavage. A principal cleavage site occurs after Asp238. Over aa 239-397, human Cytokeratin 18 shares 86% aa sequence identity with mouse Cytokeratin 18.

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