Human Ki-67/MKI67 Antibody

Antigen Affinity-purified Polyclonal Sheep IgG
Catalog Number: AF7617

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human Ki-67/MKI67 in direct ELISAs. In direct ELISAs, approximately 10% cross-reactivity with recombinant mouse Ki-67/MKI67 is observed.
Source	Polyclonal Sheep IgG
Purification	Antigen Affinity-purified
Immunogen	*E. coli*-derived recombinant human Ki-67/MKI67 Asn3120-Ile3256 Accession # P46013
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.

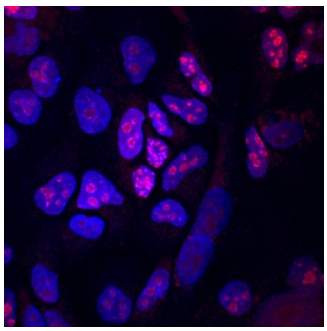
APPLICATIONS

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
Immunocytochemistry	5-15 µg/mL	See Below
Immunohistochemistry	5-15 µg/mL	See Below
Knockout Validated	Ki-67/MKI67 is specifically detected in HeLa human cervical epithelial carcinoma parental cell line but is not detectable in Ki-67/MKI67 knockout HeLa cell line.	

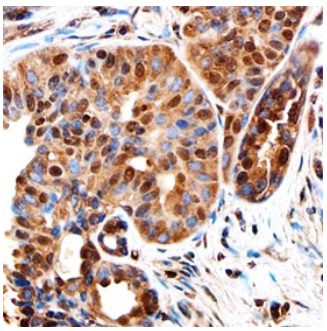
DATA

Immunocytochemistry



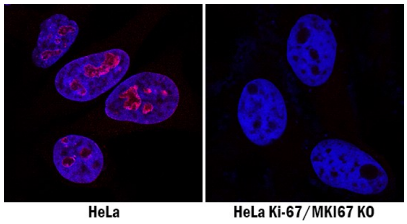
Ki-67/MKI67 in A549 Human Cell Line. Ki-67/MKI67 was detected in immersion fixed A549 human lung carcinoma cell line using Sheep Anti-Human Ki-67/MKI67 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF7617) at 5 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Sheep IgG Secondary Antibody (red; Catalog # NL010) and counterstained with DAPI (blue). Specific staining was localized to nuclei and nucleoli. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Immunohistochemistry



Ki-67/MKI67 in Human Breast Cancer Tissue. Ki-67/MKI67 was detected in immersion fixed paraffin-embedded sections of human breast cancer tissue using Sheep Anti-Human Ki-67/MKI67 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF7617) at 1 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 the nuclei of epithelial cells. View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.

Knockout Validated



Ki-67/MKI67 Specificity is Shown by Immunocytochemistry in Knockout Cell Line. Ki-67/MKI67 was detected in immersion fixed HeLa human cervical epithelial carcinoma cell line but is not detected in Ki-67/MKI67 knockout (KO) HeLa cell line using Sheep Anti-Human Ki-67/MKI67 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF7617) at 1 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 nuclei. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

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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.

BACKGROUND

MKI67 (also Ki-67) is a 350-400 kDa nuclear protein that belongs to a molecular group comprised of mitotic chromosome-associated proteins. Ki-67 was originally recognized as an antigen associated with the monoclonal Ki-67 antibody raised against Hodgkin's lymphoma nuclear material. Ki-67 is contextually expressed, being potentially found in all cells that are not in the Go phase of the cell cycle. Thus, MKI67 qualifies as a cell proliferation marker. Functionally, Ki-67 is known to interact with 160 kDa Hklp2, a protein that promotes centrosome separation and spindle bipolarity. It also directly interacts with NIFK, and apparently binds to UBF, thus playing a role in rRNA synthesis. Human MKI67 is 3256 amino acids (aa) in length. It contains one FHA domain (aa 8-98), followed by at least 24 utilized Ser/Thr phosphorylation sites and sixteen 120 aa repeats (aa 1000-2928) that are interspersed with at least 90 additional utilized phosphorylation sites. There are two potential isoform variants. One isoform is 315-345 kDa in size and shows a deletion of aa 136-495, while a second isoform contains a 58 aa substitution for aa 1-513. Over aa 3120-3256, human Ki-67 shares 46% aa sequence identity with the mouse ortholog to Ki-67.

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