Human Ki67/MKI67 Antibody

Recombinant Monoclonal Rabbit IgG Clone # 1297A
Catalog Number: MAB7617

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human Ki67/MKI67 in direct ELISAs.
Source	Recombinant Monoclonal Rabbit IgG Clone # 1297A
Purification	Protein A or G purified from cell culture supernatant
Immunogen	Human/Mouse Ki67/MKI67 synthetic peptide Accession # E9PVX6
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.

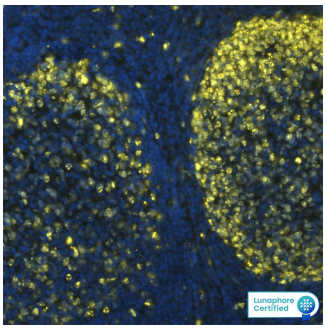
APPLICATIONS

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
Dual RNAscope ISH-IHC Compatible	5-25 µg/mL	Formalin-fixed paraffin-embedded tissue sections of human breast cancer
Immunocytochemistry	0.3-25 µg/mL	See Below
Multiplex Immunofluorescence	0.15 - 10 µg/mL	Immersion fixed paraffin-embedded sections of human tonsil and mouse intestine
Immunohistochemistry	3-25 µg/mL	See Below
Intracellular Staining by Flow Cytometry	0.25 µg/10^6 cells	See Below
Simple Western	20 µg/mL	HeLa human cervical epithelial carcinoma cell line and MCF-7 human breast cancer cell line
Knockout Validated	Ki67/MKI67 is specifically detected in Hela human cervical epithelial carcinoma parental cell line but is not detectable in Ki67/MKI67 knockout HeLa cell line.	

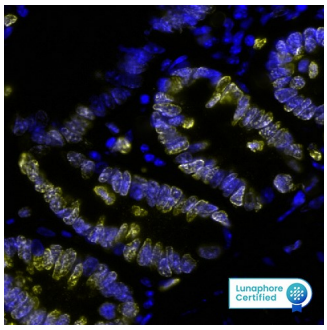
DATA

Multiplex Immunofluorescence



Detection of Ki67/MKI67 in Human Tonsil via Multiplex Immunofluorescence staining on COMET™ Ki67/MKI67 was detected in immersion fixed paraffin-embedded sections of human tonsil using Rabbit Anti-Human Ki67/MKI67 Monoclonal Antibody (Catalog # MAB7617) at 10 µg/mL at 37 ° Celsius for 4 minutes. Before incubation with the primary antibody, tissue underwent an all-in-one dewaxing and antigen retrieval preprocessing using PreTreatment Module (PT Module) and Dewax and HIER Buffer H (pH 9). Tissue was stained using the Alexa Fluor™ Plus 555 Goat anti-Rabbit IgG Secondary Antibody at 1:100 at 37 ° Celsius for 2 minutes. (Yellow; Lunaphore Catalog # DR555RB) and counterstained with DAPI (blue; Lunaphore Catalog # DR100).. Specific staining was localized to the nucleus. Protocol available in COMET™ Panel Builder.

Multiplex Immunofluorescence



Detection of Ki67 in Mouse Intestine via seqIF™ staining on COMET™ Ki67 was detected in immersion fixed paraffin-embedded sections of mouse Intestine using Rabbit Anti-Mouse Ki67, Monoclonal Antibody (Catalog # MAB7617) at 0.15ug/mL at 37° Celsius for 2 minutes. Before incubation with the primary antibody, tissue underwent an all-in-one dewaxing and antigen retrieval preprocessing using PreTreatment Module (PT Module) and Dewax and HIER Buffer H (pH 9; Epredia Catalog # TA-999-DHBH). Tissue was stained using the Alexa Fluor™ Plus 555 Goat anti-Rabbit IgG Secondary Antibody at 1:100 at 37 ° Celsius for 2 minutes. (Yellow; Lunaphore Catalog # DR555RB) and counterstained with DAPI (blue; Lunaphore Catalog # DR100). Specific staining was localized to the nucleus. Protocol available in COMET™ Panel Builder.

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Human Ki67/MKI67 Antibody

Recombinant Monoclonal Rabbit IgG Clone # 1297A
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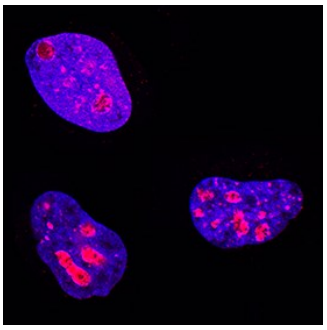
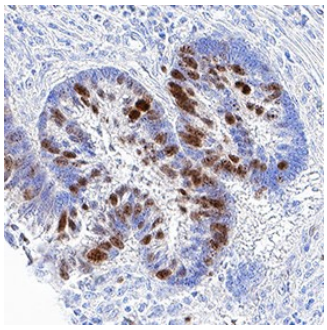
Immunocytochemistry



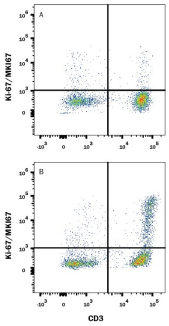
Ki67/MKI67 in HeLa Human Cell Line. Ki67/MKI67 was detected in immersion fixed HeLa human cervical epithelial carcinoma cell line using Rabbit Anti-Human Ki67/MKI67 Monoclonal Antibody (Catalog # MAB7617) at 0.3 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Rabbit IgG Secondary Antibody (red; Catalog # NL004) and counterstained with DAPI (blue). Specific staining was localized to nuclei. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Immunohistochemistry



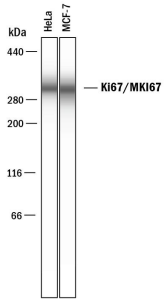
Ki67/MKI67 in Human Pancreatic Cancer Tissue. Ki67/MKI67 was detected in immersion fixed paraffin-embedded sections of human pancreatic cancer tissue using Rabbit Anti-Human Ki67/MKI67 Monoclonal Antibody (Catalog # MAB7617) at 3 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Rabbit IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC003). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to nuclei. View our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

Intracellular Staining by Flow Cytometry



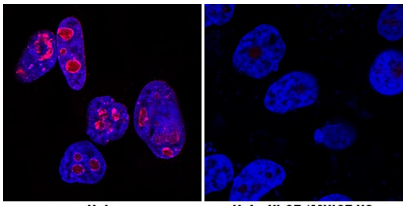
Detection of Ki67/MKI67 in Human PBMCs by Flow Cytometry. Human peripheral blood mononuclear cells (PBMCs) either A) untreated or (B) treated with 5 µg/mL PHA for 5 days were stained with Rabbit Anti-Human Ki67/MKI67 Monoclonal Antibody (Catalog # MAB7617) followed by Phycoerythrin-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # F0110) and Mouse Anti-Human CD3e APC-conjugated Monoclonal Antibody (Catalog # FAB100A). Quadrant markers were set based on control antibody staining (Catalog # MAB1050). 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.

Simple Western



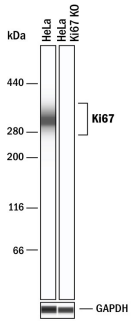
Detection of Human Ki67/MKI67 by Simple Western™. Simple Western lane view shows lysates of HeLa human cervical epithelial carcinoma cell line and MCF-7 human breast cancer cell line, loaded at 0.2 mg/mL. A specific band was detected for Ki67/MKI67 at approximately 320 kDa (as indicated) using 20 µg/mL of Rabbit Anti-Human Ki67/MKI67 Monoclonal Antibody (Catalog # MAB7617) . This experiment was conducted under reducing conditions and using the 66-440 kDa separation system.

Knockout Validated



Ki67/MKI67 Specificity is Shown by Immunocytochemistry in Knockout Cell Line. Ki67/MKI67 was detected in immersion fixed HeLa human cervical epithelial carcinoma cell line but is not detected in Ki67/MKI67 knockout (KO) HeLa cell line using Rabbit Anti-Human Ki67/MKI67 Monoclonal Antibody (Catalog # MAB7617) at 1 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 nuclei. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Knockout Validated



Human Ki67/MKI67 Specificity Shown by Simple Western™ in Knockout Cell Line. Simple Western lane view shows lysates of HeLa human cervical epithelial carcinoma cell line and Ki67 knockout HeLa cell line (KO), loaded at 0.2 mg/mL. A specific band was detected for Ki67/MKI67 at approximately 320 kDa (as indicated) using 20 µg/mL of Rabbit Anti-Human Ki67/MKI67 Monoclonal Antibody (Catalog # MAB7617). GAPDH (Catalog # MAB5718) is shown as a loading control. This experiment was conducted under reducing conditions and using the 66-440 kDa separation system.

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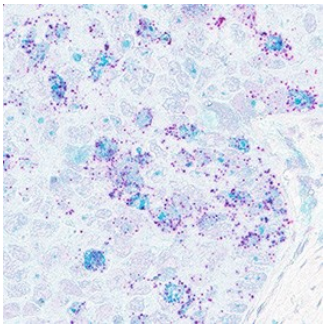
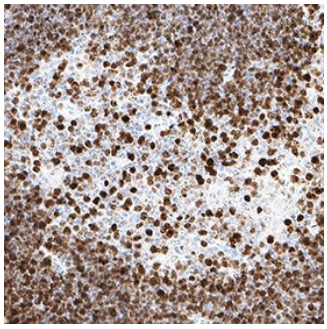
Dual RNAscope ISH-IHC Compatible



Ki-67/MKI67 in human breast cancer using Dual RNAscope®ISH and IHC. MKi67 mRNA (red) and MKi67 protein (green) were detected in formalin-fixed paraffin-embedded tissue sections of human breast cancer. ACD's Integrated Co-Detection Workflow was performed using ACD RNAScope Probe Hs-MKI67 (Catalog # 591771) and rabbit anti-human Ki67/MKI67 recombinant monoclonal antibody (Catalog # MAB7616) at 10 µg/mL. Tissue was stained using RNAscope® 2.5 HD Detection Kit-RED (Catalog # 322360) and RNAscope® 2.5 LS Green Accessory Pack (Catalog # 322550). Tissue was counterstained with 50% hematoxylin (blue).

Immunohistochemistry



Detection of Ki67/MKI67 in Mouse Thymus. Ki67/MKI67 was detected in immersion fixed paraffin-embedded sections of mouse thymus using Rabbit Anti-Human Ki67/MKI67 Monoclonal Antibody (Catalog # MAB7617) at 0.5 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 VisUCyte Antigen Retrieval Reagent-Basic (Catalog # VCTS021). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to the nucleus. View our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 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

MKI67 (also Ki67) is a 350-400 kDa nuclear protein that belongs to a molecular group comprised of mitotic chromosome-associated proteins. Ki67 was originally recognized as an antigen associated with the monoclonal Ki67 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, Ki67 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 Ki67 shares 46% aa sequence identity with the mouse ortholog to Ki67.

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