

Ki-67 Monoclonal Antibody (20Raj1), eFluor™ 660, eBioscience™

Product Details

Size	100 µg
Species Reactivity	Dog, Human
Host/Isotype	Mouse / IgG1, kappa
Recommended Isotype Control	Mouse IgG1 kappa Isotype Control (P3.6.2.8.1), eFluor™ 660, eBioscience™
Class	Monoclonal
Type	Antibody
Clone	20Raj1
Conjugate	eFluor™ 660
Excitation/Emission Max	651/668 nm
Form	Liquid
Concentration	0.2 mg/mL
Purification	Affinity chromatography
Storage buffer	PBS, pH 7.2
Contains	0.09% sodium azide
Storage conditions	4° C, store in dark, DO NOT FREEZE!
RRID	AB_2574237

Applications	Tested Dilution	Publications
Immunohistochemistry (IHC)	-	1 Publication
Immunohistochemistry (Paraffin) (IHC (P))	2 µg/mL	-
Immunocytochemistry (ICC/IF)	20 µg/mL	2 Publications
Flow Cytometry (Flow)	20 µg/mL	7 Publications

Product Specific Information

Description: The monoclonal antibody 20Raj1 recognizes the human Ki-67 protein. Two isoforms of Ki-67 exist, a 345 and 395 kDa form that are expressed in dividing cells. Ki-67 is expressed in all cell types and is detectable during active phases of the cell cycle (G1, S, G2, and mitosis) but is absent from resting cells (G0). During interphase, Ki-67 expression is localized to the nucleus but redistributes to the chromosomes during mitosis and has specifically been found to associate with heterochromatin-bound proteins such as chromobox protein homolog 3 (CBX3). In studies of tumor cells, Ki-67 expression has been used as a marker for determining the fraction of proliferating cells within a given population of tumor cells.

This monoclonal antibody 20Raj1 recognizes canine Ki-67.

Applications Reported: This 20Raj1 antibody has been reported for use in intracellular staining followed by flow cytometric analysis, microscopy, immunohistochemical staining, and immunocytochemistry.

Applications Tested: This 20Raj1 antibody has been tested by immunocytochemistry on formaldehyde-fixed and permeabilized HeLa cells, immunohistochemistry on FFPE human skin using low pH antigen retrieval, and flow cytometry on stimulated normal human peripheral blood cells using the Fcγ3/Transcription Factor Staining Buffer Set (cat. 00-5523) and protocol. For immunocytochemistry immunohistochemistry, this can be used at less than or equal to 20 µg/mL. For flow cytometry, this antibody can be used at less than or equal to 0.125 µg/test. A test is defined as the amount (µg) of antibody that will stain a cell sample in a final volume of 100 µL. Cell number should be determined empirically but can range from 10⁴ to 10⁸ cells/test. It

is recommended that the antibody be carefully titrated for optimal performance in the assay of interest.

eFluor® 660 is a replacement for Alexa Fluor® 647. eFluor® 660 emits at 659 nm and is excited with the red laser (633 nm). Please make sure that your instrument is capable of detecting this fluorochrome.

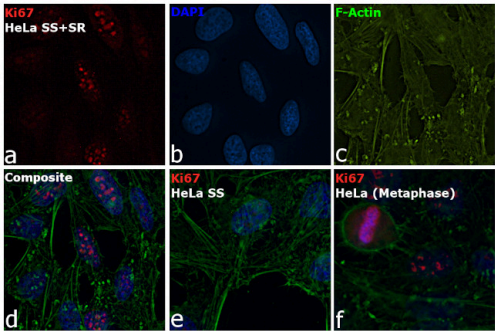
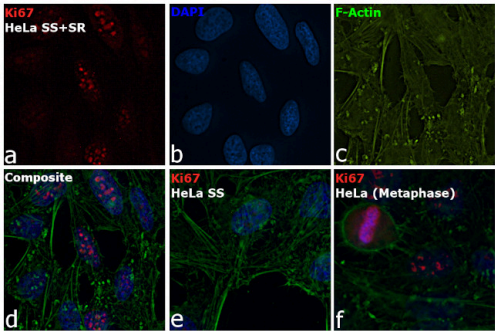
Excitation: 633-647 nm; Emission: 668 nm; Laser: Red Laser.

Filtration: 0.2 µm post-manufacturing filtered.

Product Images For Ki-67 Monoclonal Antibody (20Raj1), eFluor™ 660, eBioscience™

Ki-67 Antibody (50-5699-82) in ICC/IF

Immunofluorescence analysis of Ki-67 Monoclonal Antibody (20Raj1), eFluor™ 660, eBioscience™ was performed using 70% confluent log phase HeLa cells serum starved (SS) for 36 hours followed by serum release (SR) for 6 hours. The cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton™ X-100 for 15 minutes, and blocked with 2% BSA for 45 minutes at room temperature. The cells were labeled with Ki-67 Monoclonal Antibody (20Raj1), eFluor™ 660, eBioscience™ (Product # 50-5699-82) at 5 µg/mL concentration in 0.1% BSA, incubated at 4 degree celsius overnight (Panel a: Red). Nuclei (Panel b:Blue) were stained with ProLong™ Diamond Antifade Mountant with DAPI (Product # P36962). F-actin (Panel c: Green) was stained with Alexa Fluor™ Plus 488 Phalloidin (Product # A12379, 1:500 dilution). Panel d represents the merged image showing speckle-like localization in the nucleus. Panel e represents serum starved cells (36 hours) having no Ki-67 expression. Panel f represents a mitotic cell among the HeLa control cells showing Ki-67 signal on the chromatin. The images were captured at 60X magnification.

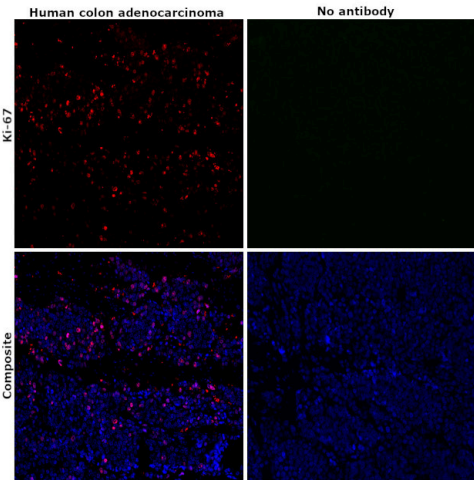


Ki-67 Antibody (50-5699-82)

Detection of upregulation of the target protein by cell treatment demonstrates antibody specificity. Immunofluorescence analysis using Ki-67 Monoclonal Antibody (SolA15), eFluor™ 660, eBioscience™ (Product # 50-5699-82), shows speckle-like localization in the nucleus in HeLa cells serum starved for 36 hours followed by serum release for 6 hours. The serum-starved HeLa cells show no signal without serum release. {TM}

Ki-67 Antibody (50-5699-82) in IHC (P)

Immunohistochemical analysis of Ki-67 was performed using formalin-fixed paraffin-embedded human colon adenocarcinoma tissue sections. To expose the target protein, heat-induced epitope retrieval was performed on de-paraffinized sections using eBioscience™ IHC Antigen Retrieval Solution - High pH (10X) (Product # 00-4956-58) diluted to 1X solution in water in a decloaking chamber at 110 degree Celsius for 15 minutes. Following antigen retrieval, the sections were blocked with 2% normal goat serum in 1X PBS for 45 minutes at room temperature and then probed with or without Ki-67 Monoclonal Antibody (20Raj1), eFluor™ 660, eBioscience™ (Product # 50-5699-82) at 2 µg/mL concentration in 0.1% normal goat serum overnight at 4 degree Celsius in a humidified chamber. ReadyProbes™ Tissue Autofluorescence Quenching Kit (Product # R37630) was used to quench autofluorescence from the tissues. Nuclei were stained with DAPI (Product # D1306) and the sections were mounted using ProLong™ Glass Antifade Mountant (Product # P36984). The images were captured on EVOS™ M7000 Imaging System (Product # AMF7000) at 20X magnification. and externally deconvoluted.



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Immunohistochemistry (1)

<p>Stem cells (Dayton, Ohio)</p> <p>Biliary tree stem cells, precursors to pancreatic committed progenitors: evidence for possible life-long pancreatic organogenesis.</p> <p>"Published figure using Ki-67 monoclonal antibody (Product # 50-5699-82) in Immunofluorescence"</p> <p>Authors: Wang Y,Lanzoni G,Carpino G,Cui CB,Dominguez-Bendala J,Wauthier E,Cardinale V,Oikawa T,Pileggi A, Gerber D,Furth ME,Alvaro D,Gaudio E,Inverardi L,Reid LM</p>	<p>Year 2013</p>
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Immunocytochemistry (2)

<p>Scientific reports</p> <p>Core Transcription Factors, MicroRNAs, and Small Molecules Drive Transdifferentiation of Human Fibroblasts Towards The Cardiac Cell Lineage.</p> <p>"Published figure using Ki-67 monoclonal antibody (Product # 50-5699-82) in Immunofluorescence"</p> <p>Authors: Christoforou N,Chakraborty S,Kirkton RD,Adler AF,Addis RC,Leong KW</p>	<p>Year 2017</p>
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<p>Arteriosclerosis, thrombosis, and vascular biology</p> <p>S100A6 Regulates Endothelial Cell Cycle Progression by Attenuating Antiproliferative Signal Transducers and Activators of Transcription 1 Signaling.</p> <p>"Published figure using Ki-67 monoclonal antibody (Product # 50-5699-82) in Immunocytochemistry"</p> <p>Authors: Lerchenmüller C,Heißenberg J,Damilano F,Beggeridis VJ,Krämer I,Bochaton-Piallat ML,Hirschberg K,Busch M, Katus HA,Peppel K,Rosenzweig A,Busch H,Boerries M,Most P</p>	<p>Year 2016</p>
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Flow Cytometry (7)

<p>Cell reports. Medicine</p> <p>Protein/AS01_B vaccination elicits stronger, more Th2-skewed antigen-specific human T follicular helper cell responses than heterologous viral vectors.</p> <p>"Published figure using Ki-67 monoclonal antibody (Product # 50-5699-82) in Flow Cytometry"</p> <p>Authors: Nielsen CM,Ogbe A,Pedroza-Pacheco I,Doeleman SE,Chen Y,Silk SE,Barrett JR,Elias SC,Miura K,Diouf A, Bardelli M,Dabbs RA,Barfod L,Long CA,Haynes BF,Payne RO,Minassian AM,Bradley T,Draper SJ,Borrow P</p>	<p>Year 2021</p>
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