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 Foxp3/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 10^5 to 10^8 cells/test. It

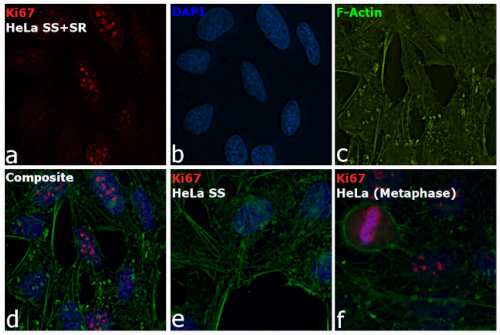
is recommended that the antibody be carefully titrated for optimal performance in the assay of interest.

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 fluorochome.

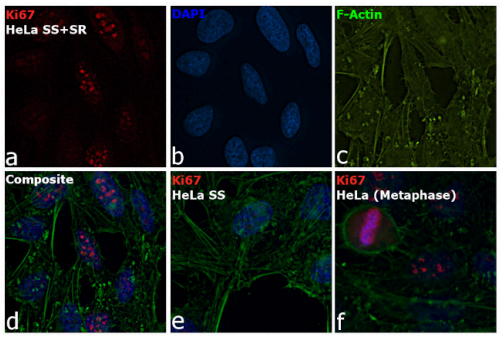
Excitation: 633-647 nm; Emission: 668 nm; Laser: Red Laser.

Filtration: 0.2 µm post-manufacturing filtered.

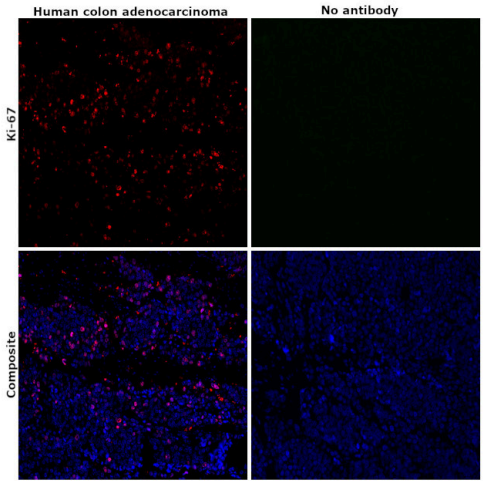
Product Images For Ki-67 Monoclonal Antibody (20Raj1), eFluor™ 660, eBioscience™



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.

View more figures on thermofisher.com

10 References

Immunohistochemistry (1)

Stem cells (Dayton, Ohio)

Year
2013

Biliary tree stem cells, precursors to pancreatic committed progenitors: evidence for possible life-long pancreatic organogenesis.

"Published figure using Ki-67 monoclonal antibody (Product # 50-5699-82) in Immunofluorescence"

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

Immunocytochemistry (2)

Scientific reports

Year
2017

Core Transcription Factors, MicroRNAs, and Small Molecules Drive Transdifferentiation of Human Fibroblasts Towards The Cardiac Cell Lineage.

"Published figure using Ki-67 monoclonal antibody (Product # 50-5699-82) in Immunofluorescence"

Authors: Christoforou N,Chakraborty S,Kirkton RD,Adler AF,Addis RC,Leong KW

Arteriosclerosis, thrombosis, and vascular biology

Year
2016

S100A6 Regulates Endothelial Cell Cycle Progression by Attenuating Antiproliferative Signal Transducers and Activators of Transcription 1 Signaling.

"Published figure using Ki-67 monoclonal antibody (Product # 50-5699-82) in Immunocytochemistry"

Authors: Lerchenmüller C,Heißenberg J,Damilano F,Bezzeridis VJ,Krämer I,Bochaton-Piallat ML,Hirschberg K,Busch M, Katus HA,Peppel K,Rosenzweig A,Busch H,Boerries M,Most P

Flow Cytometry (7)

Cell reports. Medicine

Year
2021

Protein/AS01$_B$ vaccination elicits stronger, more Th2-skewed antigen-specific human T follicular helper cell responses than heterologous viral vectors.

"Published figure using Ki-67 monoclonal antibody (Product # 50-5699-82) in Flow Cytometry"

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

View more Flow references on thermofisher.com

More applications with references on thermofisher.com