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Ki-67 Recombinant Polyclonal Antibody (12HCLC)

Catalog Number **710229**

Product data sheet

Details	
Size	100 µg
Host/Isotope	Rabbit / IgG
Class	Recombinant Polyclonal
Type	Antibody
Clone	12HCLC
Immunogen	Peptide corresponding to amino acids 1213-1232 of human Ki-67
Conjugate	Unconjugated
Form	Liquid
Concentration	0.5 mg/mL
Purification	Protein A
Storage buffer	PBS
Contains	0.09% sodium azide
Storage Conditions	Store at 4°C short term. For long term storage, store at -20°C, avoiding freeze/thaw cycles.

Species Reactivity	
Species reactivity	Human

Tested Applications	Dilution *
ChIP assay (ChIP)	1 µL
Immunocytochemistry (ICC/IF)	1-3 µg/mL

* Suggested working dilutions are given as a guide only. It is recommended that the user titrate the product for use in their own experiment using appropriate negative and positive controls.

Product specific information

This antibody is predicted to react with non-human primate, mouse and rat based on sequence homology. Recombinant rabbit polyclonal antibodies are unique offerings from Thermo Fisher Scientific. They are comprised of a selection of multiple different recombinant monoclonal antibodies, providing the best of both worlds - the sensitivity of polyclonal antibodies with the specificity of monoclonal antibodies - all delivered with the consistency only found in a recombinant antibody. While functionally the same as a polyclonal antibody - recognizing multiple epitope sites on the target and producing higher detection sensitivity for low abundance targets - a recombinant rabbit polyclonal antibody has a known mixture of light and heavy chains. The exact population can be produced in every lot, circumventing the biological variability typically associated with polyclonal antibody production.

Background/Target Information

Ki-67 is a nuclear protein that is expressed during various stages in the cell cycle, particularly during late G1, S, G2, and M phases. The protein has a forkhead associated domain (FHA) through which it associates with euchromatin at the perichromosomal layer, the centromeric heterochromatin, and the nucleolus. Ki-67 is shown to have a cell cycle dependent topographical distribution with perinucleolar expression at G1, expression in the nuclear matrix at G2, and expression on the chromosomes during M phase. Ki-67 is commonly used as a proliferation marker because it is not detected in G0 cells, but increases steadily from G1 through mitosis. Ki-67 antibodies are useful in establishing the cell growing fraction in neoplasms. In neoplastic tissues, the prognostic value is comparable to the tritiated thymidine-labelling index. The correlation between low Ki-67 index and histologically low-grade tumors is strong. Ki-67 is routinely used as a neuronal marker of cell cycling and proliferation.

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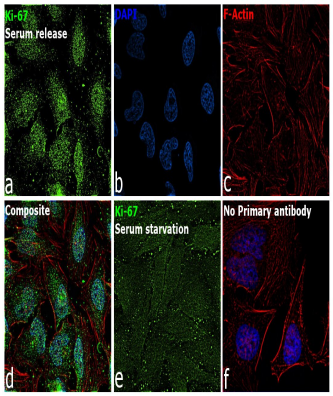
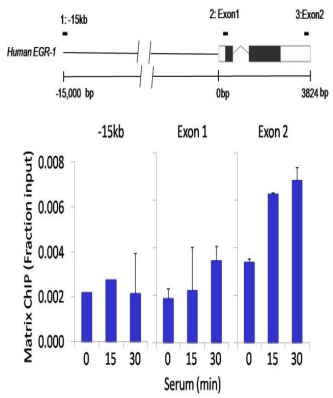
Product Images For Ki-67 Recombinant Polyclonal Antibody (12HCLC)



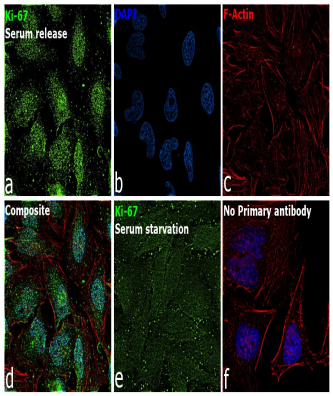
Ki-67 Antibody (710229)

Antibody specificity was demonstrated by detection of differential basal expression of the target across cell models owing to their inherent genetic constitution. Immunofluorescence analysis using Anti-Ki-67 Recombinant Polyclonal Antibody (12HCLC) (Product # 710229), shows higher expression of Ki-67 in serum released HeLa cells as compared to Serum starved HeLa cells. {RE}



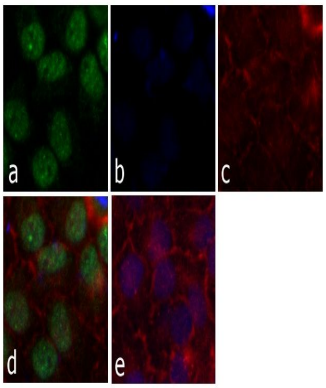
Ki-67 Antibody (710229) in ChIP

Chromatin immunoprecipitation analysis of Ki-67 was performed using cross-linked chromatin from 1 x 10^6 HCT116 human colon carcinoma cells treated with serum for 0, 15, and 30 minutes. Immunoprecipitation was performed using a multiplex microplate Matrix ChIP assay (see reference for Matrix ChIP protocol: http://www.ncbi.nlm.nih.gov/pubmed /22098709) with 1.0 µL/100 µL well volume of a Ki-67 Recombinant Rabbit Polyclonal Antibody (Product # 710229). Chromatin aliquots from ~1 x 10^5 cells were used per ChIP pull-down. Quantitative PCR data were done in quadruplicate using 1 µL of eluted DNA in 2 µL SYBR real-time PCR reactions containing primers to amplify -15kb upstream of the human Egr-1 locus, or exon-1 or exon-2 of Egr-1. PCR calibration curves were generated for each primer pair from a dilution series of sheared total genomic DNA. Quantitation of immunoprecipitated chromatin is presented as signal relative to the total amount of input chromatin. Results represent the mean +/- SEM for three experiments. A schematic representation of the Egr-1 locus is shown above the data where boxes represent exons (black boxes = translated regions, white boxes = untranslated regions), the zigzag line represents an intron, and the straight line represents upstream sequence. Regions amplified by Egr-1 primers are represented by black bars. Data courtesy of the Innovators Program.



Ki-67 Antibody (710229) in ICC/IF

Immunofluorescence analysis of Proliferation marker protein Ki-67 was performed using 70% confluent log phase HeLa cells (Serum release,8 Hrs). 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 Recombinant Polyclonal Antibody (12HCLC) (Product # 710229) at 1:100 dilution in 0.1% BSA, incubated at 4 degree celsius overnight and then labeled with Goat anti-Rabbit IgG (Heavy Chain) Superclonal™ Recombinant Secondary Antibody, Alexa Fluor® 488 conjugate (Product # A27034), (1:2000 dilution), for 45 minutes at room temperature (Panel a: Green). Nuclei (Panel b: Blue) were stained with ProLong™ Diamond Antifade Mountant with DAPI (Product # P36962). F-actin (Panel c: Red) was stained withRhodamine Phalloidin (Product # R415, 1:300 dilution). Panel d represents the merged image showing nuclear localization. Panel e represents HeLa (serum starved for 16Hrs). Panel f represents control cells with no primary antibody to assess background. The images were captured at 60X magnification.



Ki-67 Antibody (710229) in ICC/IF

Immunofluorescent analysis of Ki-67 was done on serum starved (16 hours) followed by 6 hours serum released HeLa cells. The cells were fixed with 4% paraformaldehyde for 15 minutes; permeabilized with 0.25% Triton X-100 for 10 minutes followed by blocking with 5% BSA for 1 hour at room temperature. The cells were incubated with Ki-67 Recombinant Rabbit Polyclonal Antibody (Product # 710229) at 1 µgmL in 1% BSA and incubated for 3 hours at room temperature and then labeled with Alexa Fluor® 488 Goat anti-Rabbit IgG Secondary Antibody (Product # A-11008) at a dilution of 1:400 for 30 minutes at room temperature (Panel a: green). Nuclei (Panel b: blue) were stained with SlowFade Gold Antifade Mountant with DAPI (Product # S36938). F-actin (Panel c: red) was stained with Alexa Fluor 594 Phalloidin (Product # A12381). Panel d is a merged image showing nuclear localization of Ki-67. Panel e shows no primary antibody control. The images were captured at 20X magnification.

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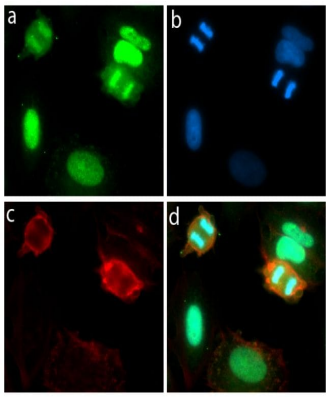

Ki-67 Antibody (710229) in ICC/IF

Immunofluorescent analysis of Ki-67 in HeLa cells (serum-starved for 36 hrs followed by serum release for 8 hrs) using a Ki-67 Recombinant Rabbit Polyclonal Antibody (Product # 710229) followed by detection using an Alexa Fluor 488-conjugated Goat anti-Rabbit secondary antibody (green) (Image A). Nuclei were stained using DAPI (Image B) and actin stained with Alexa Fluor 594 phalloidin (red) (image C). Image D is a composite image showing nuclear localization of Ki-67.

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