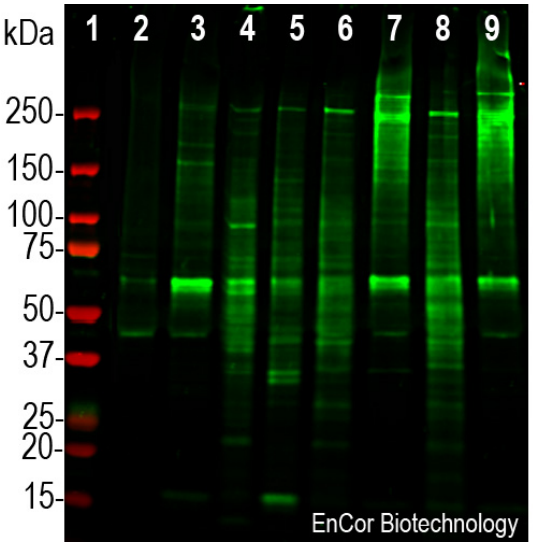
Ki67, KI-67 in rodent Rabbit Polyclonal Antibody

RPCA-Ki67-Ro

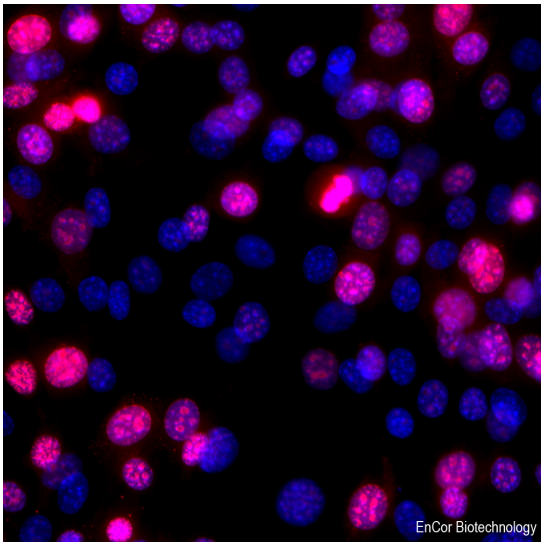
Ordering Information
Web *www.encorbio.com*
Email *admin@encorbio.com*
Phone *352-372-7022*
Fax *352-372-7066*

HGNC Name: *MKI67*
UniProt: *E9PVX6*
RRID: *AB_2861219*
Immunogen: *Recombinant segment of mouse sequence expressed in and purified from E. coli.*
Format: *Supplied as an aliquot of serum plus 5mM sodium azide*
Storage: *Storage for short term at 4°C recommended, for longer term at -20°C, minimize freeze/thaw cycles*
Recommended dilutions:
WB: 1:5,000-10,000. IF 1:2,000-5,000, IHC 1:1,000

Applications	Host	Isotype	Molecular Wt.	Species Cross-Reactivity
WB, IF/ICC, IHC	Rabbit		345kDa, 395kDa	Rt, Ms



Western blot analysis of cytosol and nuclear fractions from different cell line lysates using rabbit pAb to Ki-67 RPCA-Ki-67-Ro, dilution 1:1,000, (green): [1] protein standard (red) and extracts of [2] HeLa cells cytosol, [3] HeLa cells nuclear fraction, [4] HEK293 cytosol, [5] HEK293 nuclear fraction, [6] NIH/3T3 cytosol, [7] NIH/3T3 nuclear fraction, [8] C6 cytosol, and [9] C6 nuclear fraction. Strong bands above 250 kDa mark correspond to two major isoforms of Ki-67 protein expressed in rodent cell lines – NIH/3T3 and rat glioma cell line C6. Smaller fragments of these isoforms are also detected on the blot in all cell lines.



Immunofluorescent analysis of mouse NIH/3T3 cells stained with rabbit pAb to Ki-67 RPCA-Ki-67-Ro, dilution 1:1,000, red. The blue is Hoechst staining of nuclear DNA. The RPCA-Ki-67-Ro antibody stains Ki-67 protein predominantly expressed in nucleoli of cells in interphase, while cells in the quiescent G0 state do not express Ki-67 protein. Dividing cells express Ki67 surrounding condensed chromosomes.

Background:

The Ki67 protein was first discovered when researchers attempted to generate cancer cell specific monoclonal antibodies by injecting mice with nuclear preparations from Hodgkin's lymphoma cells (1). They obtained a monoclonal antibody which recognized two large proteins of apparent molecular weight 345kDa and 395kDa. The clone was named Ki67 after Kiel, Germany where the original work was done and the number of the 96 well plate in which the clone was found. The two proteins were found to be heavily expressed in proliferating cells, but to be absent in quiescent cells, and later work showed that they were the product of a single gene. The presence of the Ki67 protein is frequently used as an indicator of cell proliferation and its level of expression is one of the most reliable biomarkers of proliferative status of cancer cells (2-5). Much research shows a correlation between Ki67 protein level and prognosis in cancer patients, when high Ki67 levels being associated with poorer outcomes (e.g. 6,7). The original Ki67 antibody and several others have become so widely used that a search for "(Ki67 or Ki-67) and antibody" in PubMed in August 2018 produced over 5,600 results. Recent studies show that Ki67 functions as a "biological surfactant", which is essential for the fidelity of separation of condensed chromosomal DNA into the two daughter cells during cell division (8). This presumably explains the highly basic nature of Ki67, allowing a charge-based interaction with nucleic acids, the lack of this protein in non-dividing cells and the relative lack of protein sequence conservation.

The RPCA-Ki67-Ro antibody was made against a recombinant construct including amino the acids 956-1,322 of the mouse sequence XP_006507475.1, a region corresponding to 2nd, 3rd and 4th Ki67 type repeats. Since the Ki67 protein is relatively poorly conserved in amino acid sequence, this antibody is not recommended for use on human tissues, for which our RPCA-Ki67 antibody, made against the human protein, would be superior. Note that the Ki67 proteins are very unstable and only expressed in large amounts in situations where many cells are dividing. As a result of the very short half life of Ki67 there are usually numerous fragments visible on western blots running below the major 395kDa and 345kDa bands.

References:

1. Gerdes J, Schwab U, Lemke H, Stein H. Production of a mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. *Int. J. Cancer 31:13-20 (1983)*.
2. Kill IR, Faragher RGA, Lawrence K. Shall S. The expression of proliferation-dependent antigens during the lifespan of normal and progeroid human fibroblasts in culture. *J. Cell Sci. 107:571-9 (1994)*.
3. Yerushalmi R, et al. Ki67 in breast cancer: Prognostic and predictive potential. *Lancet Oncol. 11:174–83 (2010)*.
4. Josefsson A, et al. Low endoglin vascular density and Ki67 index in Gleason score 6 tumours may identify prostate cancer patients suitable for surveillance. *Scand. J. Urol. Nephrol. 46:247–57 (2012)*.
5. Ishihara M, et al. Retrospective analysis of risk factors for central nervous system metastases in operable breast cancer: effects of biologic subtype and Ki67 overexpression on survival. *Oncology. 84:135–140 (2013)*.
6. Cheang MC, et al. Ki67 Index, HER2 Status, and Prognosis of Patients With Luminal B Breast Cancer. *J. Natl. Cancer Inst. 101:736-50 (2009)*.
7. Margulis V, et al. Multi-institutional validation of the predictive value of Ki-67 labeling index in patients with urinary bladder cancer. *J. Natl. Cancer Inst. 101:114-9 (2009)*.
8. Cuylen S, et al. Ki-67 acts as a biological surfactant to disperse mitotic chromosomes.*Nature. 535:308-12 (2016)*.

Abbreviation Key:

mAb—Monoclonal Antibody pAb—Polyclonal Antibody WB—Western Blot IF—Immunofluorescence ICC—Immunocytochemistry IHC—Immunohistochemistry E—ELISA Hu—Human Mo—Monkey Do—Dog Rt—Rat Ms—Mouse Co—Cow Pi—Pig Ho—Horse Ch—Chicken Dr—D. rerio Dm—D. melanogaster Sm—S. mutans Ce—C. elegans Sc—S. cerevisiae Sa—S. aureus Ec—E. coli.

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