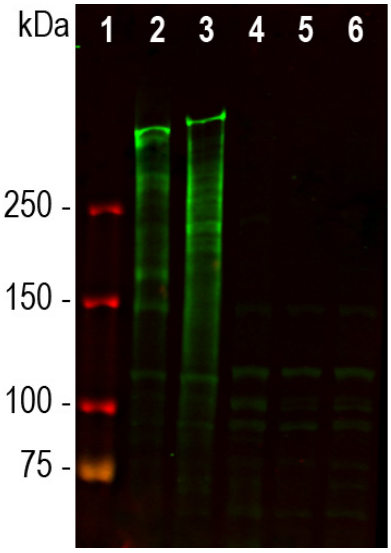
Ki67, KI-67
Mouse Monoclonal Antibody

MCA-5F86

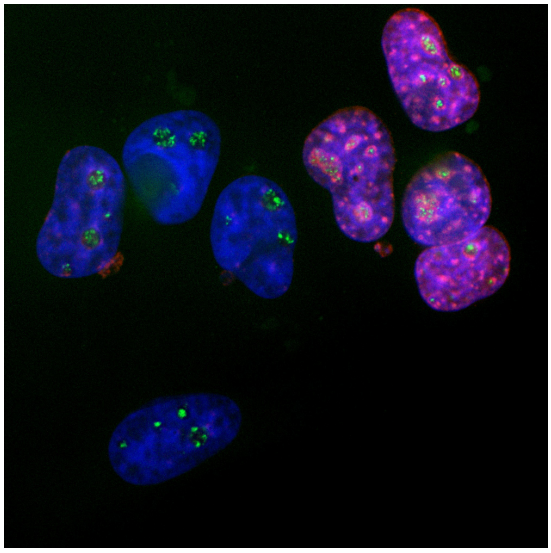
Ordering Information
Web ***www.encorbio.com***
Email ***admin@encorbio.com***
Phone ***352-372-7022***
Fax ***352-372-7066***

HGNC Name: *MKI67*
UniProt: *E9PVX6*
RRID: *AB_2861220*
Immunogen: *Recombinant construct containing amino acids 956-1322 of the mouse sequence XP_006507475 expressed in and purified from E. coli.*
Format: *Purified antibody at 1mg/mL in 50% PBS, 50% glycerol plus 5mM NaN_3*
Storage: *Storage for short term at 4°C recommended, for longer term at -20°C, minimize freeze/thaw cycles*
Recommended dilutions:
WB: 1:1,000-2,000. IF 1:2,000-5,000, IHC 1:1,000

Applications	Host	Isotype	Molecular Wt.	Species Cross-Reactivity
WB, IF/ICC, IHC	Mouse	IgG1	345kDa, 395kDa	Rt, Ms



Western blot analysis of cell lysates from various cell lines using mouse mAb to rodent forms of Ki67, MCA-5F86, dilution 1:1,000, (green): [1] protein standard (red), and extracts of mouse NIH/3T3 [2], rat glioma C6 [3] cells. Strong bands above 250kDa correspond to two major isoforms of Ki67 protein expressed in the rodent cell lines and smaller fragments of these isoforms are also detected on the blot. We also tested extracts of human cell lines, HEK293 [4], HeLa [5] and SH-SY5Y cells [6]. This antibody binds an epitope not conserved in human cells and so fails to stain these extracts.



Immunofluorescent analysis of HeLa cells stained with rabbit pAb to Ki67 RPCA-Ki67, dilution 1:5,000 in red, and mouse monoclonal antibody to fibrillarin, MCA-38F3, dilution 1:2,000, in green. The blue is DAPI staining of nuclear DNA. The Ki67 protein accumulates in and around the nucleoli of interphase cells such as those on the right, and the nucleoli are revealed by the fibrillarin antibody. In contrast, cells in the quiescent G0 state such as those on the left are Ki67 negative but fibrillarin positive.

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 MCA-5F86 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:

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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).
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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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