

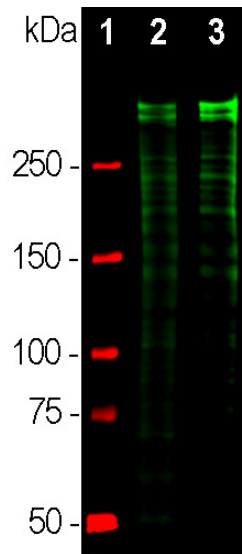
Ordering Information
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HGNC Name: MKI67
UniProt: P46013
RRID: AB_2637050
Immunogen: Recombinant human construct containing amino acids 1,111-1,490 expressed in and purified from *E. coli*.
Format: Supplied as an aliquot of serum plus 5mM sodium azide
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

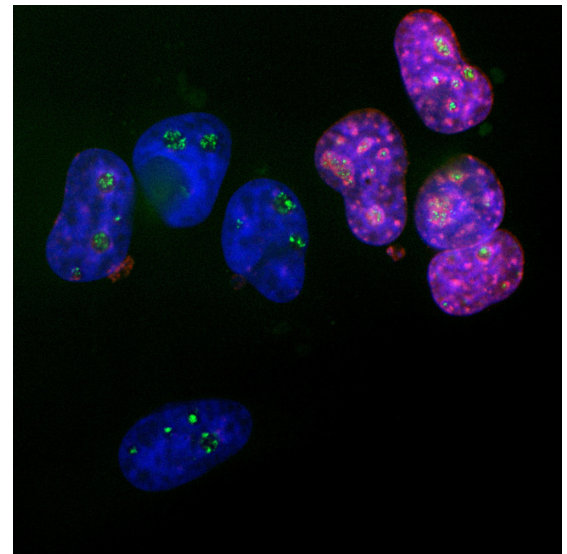
References:

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3. Yerushalmi R, et al. Ki67 in breast cancer: Prognostic and predictive potential. *Lancet Oncol.* 11:174-83 (2010).
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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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8. Cuylen S, et al. Ki-67 acts as a biological surfactant to disperse mitotic chromosomes. *Nature.* 535:308-12 (2016).

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



Western blot analysis of equal amounts of cell lysates using rabbit pAb to Ki67 RPCA-Ki67, dilution 1:10,000, (green): [1] protein standard (red), [2] rapidly growing HeLa cell cultures, [3] rapidly growing HEK293 cell cultures. Strong double bands larger than the 250kDa standard correspond to full length 345kDa and 395kDa Ki67 isoforms, while smaller proteolytic fragments of these isoforms are also invariably detected on the blot.



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 CPCA-Ki67 was made against a recombinant construct including amino acids 1,111-1,490 of the human sequence P46013, a region corresponding to 2nd, 3rd and 4th Ki67 type repeats. Although Ki67 is relatively poorly conserved in amino acid sequence, this antibody recognizes both rat and mouse Ki67 and also works very well on formalin fixed and paraffin embedded human and rodent sections. 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 395kDa and 345kDa bands.

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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*.