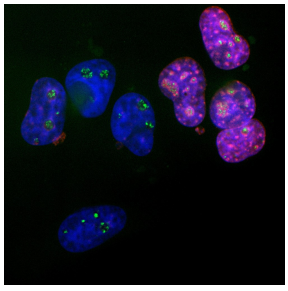
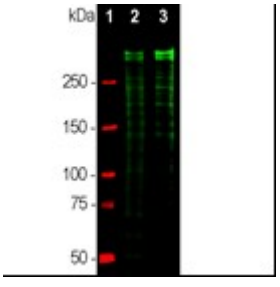
Product Data Sheet: Ki-67

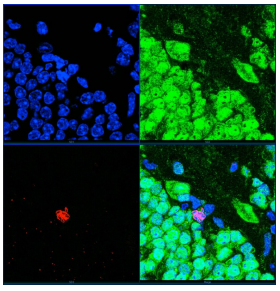
Cat. No:	MAB-90948
Conjugate:	Unconjugated
Size:	100 ug
Clone:	SP6
Concentration:	1mg/ml
Host:	Rabbit
Isotype:	IgG
Immunogen:	SRecombinant human construct containing amino acids 1,111-1,490 expressed in and purified from E. coli.
Reactivity:	Hu, Ms, Rt
Applications:	Western Blot : 1:2,000-1:5,000 Immunohistochemsitry (paraffin, formalin, frozen Tissues):1:500 Immunofluorescence: 1:1,000-1:2,500 Immunocytochemistry: 1:1,000-1:2,500
Molecular Weight:	345 kDa – 395 kDa
Purification:	Serum
Background:	The Ki-67 proteins were first discovered in an attempt to generate cancer specific monoclonal antibodies. A monoclonal antibody which bound to structures in the nuclei of dividing but not quiescent cells was produced and shown to bind two very large proteins of molecular weight 345kDa and 395kDa. The two proteins were derived from alternate transcripts of a single gene. The presence of Ki-67 proteins, detected with an appropriate antibody, is an indicator of cell proliferation and the level of Ki-67 expression is one of the most reliable biomarkers of proliferative status of cancer cells. The Ki-67 antibody was raised against a recombinant construct containing amino acids 1,111-1,490 of human Ki-67 isotype 1. The antibody can be used to identify dividing cells in rat and mouse brain and also works on paraffin sections of human tissues, where it is useful to identify cancer cells. Mouse select image at left for larger view.
Form:	Liquid
Buffer:	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



Immunofluorescent analysis of HeLa cells stained with rabbit Anti-Ki-67 dilution 1:2,500 in red, and mouse



Western blot analysis of equal amounts of cell lysates using rabbit Anti-Ki-67 Ki-67, dilution 1:5,000, (green): [1]

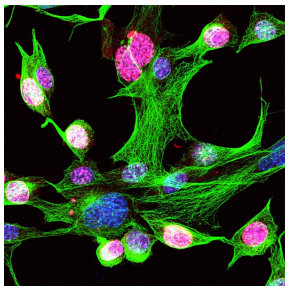


High magnification confocal image of adult mouse hippocampus dentate region stained with Anti-Ki67 Monoclonal

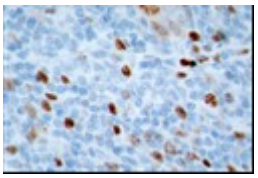
monoclonal antibody to Fibrillarin, dilution 1:2,000, in green. The blue is DAPI staining of nuclear DNA.

protein standard (red), [2] HeLa cells, [3] HEK293 cells. Strong double bands above 250kDa correspond to the two major Ki-67 isoforms of molecular weight 345kDa and 395kDa. Smaller proteolytic fragments of these isoforms are also detected on the blot.

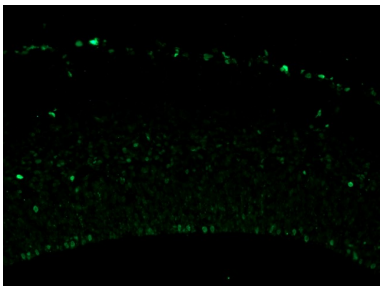
1:2,000 – in green. Blue is Hoechst dye staining of DNA. Top left is DNA, top right FOX3/NeuN, bottom left Ki-67 and bottom right all three merged. Dividing cells are very rare in adult animals, but one can be seen in the center of the image. Chromosomes can be seen in blue and their Ki-67 coating can be seen in red. The dividing cell is FOX3/NeuN negative and so is presumably a glial cell.



Mouse NIH-3T3 cells stained with Anti-Ki-67 1:2,500 in red and mouse mAb to β-tubulin, 1:1,000 in green. The Ki-67 strongly stains the nuclei of dividing cells, but not quiescent cells



A section of human breast tissue including both normal and cancer cells. The cancer cells divide rapidly and heavily express Ki-67 and so stain strongly with the Anti-Ki-67 antibody.



Mouse embryonic brain tissue of neural cortex Immunohistochemistry on Frozen Tissue (1:100) Dr. Vania Broccoli – Dr.ssa Dell'Anno – Dr. Massimino Stem Cells & Neurogenesis Unit – HSR (MI)

IHC (Frozen) PROTOCOL

1) Extract tissues in cold 1X PBS
2) Fix the tissues in 4% PFA overnight
3) Incubate the tissues in 30% sucrose as long as these tissues are not placed on the bottom.
4) Include them in OCT
5) Cut them into 10 um slices with a cryostat .

1) Wash the slides once in 1X PBS,
2) Antigen Retrieval : Boil the slides in 10 mM sodium citrate,
3) Incubate the slides 1 hour in blocking solution (10% GS, 0.3% triton in 1X PBS) at RT,
Incubate them overnight with the primary antibody diluted 1:100 in blocking solution at 4°C,
4) Wash the slides 5 times in 1X PBS,
5) Incubate the slides 1 hour with the secondary antibody (alexa) diluted 1:500 in blocking solution at RT,
6) Wash the slides 5 times in 1X PBS , and mount them.

STORAGE & STABILITY

Store at 2-8°C. Do not freeze. The user must validate any other storage conditions. When properly stored, the reagent is stable to the date indicated on the label. Do not use the reagent beyond the expiration date.
There are no definitive signs to indicate instability of this product; therefore, positive and negative controls should be tested simultaneously with unknown specimens.

WARNINGS & PRECAUTIONS:
1. Avoid contact of reagents with eyes and mucous membranes. If reagents come into contact with sensitive areas, wash with copious amounts of water.
2. This product is harmful if swallowed.
3. Consult local or state authorities with regard to recommended method of disposal.
4. Avoid microbial contamination of reagents.