



FLUOROCROME, LLC

5285 East Sixth Avenue
Denver, CO 80220
Telephone (303) 394-1000

Email: info@Fluorochrome.com
website: www.Fluorochrome.com

Ki-67 Antibody-W

Ki67 is an intrinsic marker of ongoing cell proliferation and is present during all active phases of the cell cycle (G1-M). This antibody is polyclonal and was raised in rabbit to the human peptide sequence – TPKEKAQALEDLAGFKELFQT – that was coupled to thyroglobulin by glutaraldehyde before being injected into the rabbit. The antibody has been used for immunocytochemistry in rat and human brain tissue. The vial contains about 200ul at a dilution of 1/100. The antibody can be further diluted to at least 1/20,000 in BSA diluent (50mM KPBS, 0.4% Triton, 1% BSA, 1% NGS). The antibody should be stored at -20C. Also included is filter paper absorbed with the specific peptide for blocking. 500ul of the diluted antibody can be added to the tube to achieve a 10 uM block. Incubate for at least 1hr at room temperature (RT) before applying to tissue. The antibody blocks specifically with this peptide and does not block with other peptides absorbed on filter paper.

Protocol:

Fresh frozen 10-30 micron sections are fixed in 4% paraformaldehyde (FA) for 1 hour at RT, washed in phosphate buffered saline (PBS), and then treated in 10% Sodium Citrate for 40 minutes at 90C. The sections are allowed to cool for 20 minutes in the sodium citrate solution, washed in PBS and incubated with 0.1 to 0.3% H₂O₂ in PBS for 10 to 30 minutes. The tissue is again washed in PBS, then treated with an Avidin/Biotin blocking kit (Vector-cat #SP-2001), washed and incubated with BSA diluent for 1 hr at RT. The Ki67 antibody (1/20,000) is applied to the tissue overnight at RT. After washing in PBS the sections are incubated in Biotinylated GAR at 1/1000 (Vector labs-cat#BA1000) for 1hr at RT, washed and then incubated in Avidin/Biotin at 1/1000 (Vector labs-cat#PK6100) for 1hr at RT. After washing the signal is visualized with Diaminobenzidine (0.04%) in 0.1M Sodium Acetate with 0.06% H₂O₂ for six minutes (Nickel Chloride at 2.5% may also be added). Sections are then water washed (counterstained if desired), dehydrated and cover slipped.

This antibody also works with the protocol used to visualize the BRDU antibody sold by Fluorochrome, LLC. 4% FA perfused tissue may be used instead of fresh frozen sections.

Publications:

Perez et al, The Journal of Neuroscience, May 13, 2009:29(19):6379-6387

Garcia-Fuster et al, in preparation: Individual differences in adult hippocampal neurogenesis following experimenter administration of cocaine.