Anti-Human Ki67 Antibody [SR00-02] - BSA and Azide free (Detector)

HA724537

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	ELISA(Det)
Clone number:	SR00-02

Description: The Ki-67 protein is a nuclear protein doublet, 345-395 kDa, playing a pivotal role in maintaining cell proliferation. Ki-67 is present in all non-G0 phases of the cell cycle. Beginning in the mid G1, the level increases through S and G2 to reach a peak in M. In the end of M, is is rapidly catabolized. The Ki-67 labelling index (LI), i.e., the percentage of cells in a tissue staining for Ki-67, indicates the growth fraction. For many tumours, the rate of cell proliferation as assessed by Ki-67 immunoreactivity correlates with tumour grade and clinical course. In Non-Hodgkin lymphoma a labelling index of less than 20% is seen in low grade lymphomas, greater than 20% is associated with high grade lymphomas. Low grade lymphomas with a labelling index in excess of 5% have a worse prognosis than those with an index of less than 5%. In Burkitt and Burkitt-like lymphoma, nearly 100% of the nuclei are stained. This can be used as a diagnostic criterion. In gliomas the indices ranges from 0% to 5% for low grade astrocytomas while anaplastic astrocytomas and glioblastomas most frequently show an index above 10%. In soft tissue sarcomas Ki-67 index is positively correlated with mitotic count, cellularity and histological grade. In some benign tumours, like meningioma, a high LI is associated with a high recurrence rate. In dysplasia in Barrett's oesophagus and in granulosa cell tumours and ovarian serous tumours, Ki-67 LI is associated with progression. In the former, reproducibility of dysplasia grading is improved when Ki67 is included. In breast cancer, the proliferative index measured by Ki67 immunoreactivity has both prognostic and predictive value.

Immunogen:	Recombinant protein within Human Ki67 aa 1,000-1,234 (HA211578).
Positive control:	Recombinant Human Ki67 protein (HA211578).
Subcellular location:	Chromosome, Nucleus, nucleolus.
Database links:	SwissProt: P46013 Human
Recommended Dilutions:	
ELISA(Det)	Use at an assay dependent concentration. Can be paired for Sandwich ELISA with Rabbit monoclonal [PSH25-46] to Human Ki67 (Capture) (HA724536) and Recombinant Human Ki67 protein (HA211578) as the standard. The reference range value is 31.3-8,000 pg/mL.
Storage Buffer:	1*PBS (pH7.4).
Storage Instruction:	Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.
Purity:	Protein A affinity purified.

Images

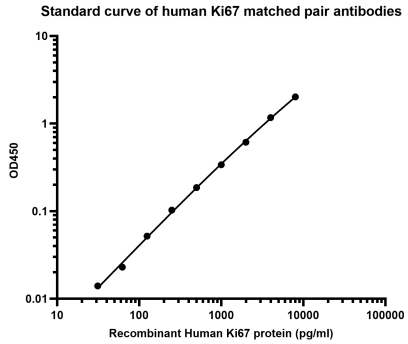

Fig1: Sandwich ELISA analysis of Human Ki67 matched pair antibodies

Capture: HA724536, Human Ki67 Rabbit mAb [PSH25-46]
Detector: HA724537, Human Ki67 Rabbit mAb [SR00-02]

Elisa assay was performed by coating wells of a 96-well plate with 100 µl per well of capture antibody (HA724536) diluted in carbonate/bicarbonate buffer, at a concentration of 5 µg/mL overnight at 4℃. Wells of the plate were washed, blocked with 150 µl 0.05% tween-20 1% BSA blocking buffer, and incubated with serial diluted Recombinant Human Ki67 protein (HA211578) starting from 8,000 pg/mL to 0 pg/mL and detect antibody (HA724537, Biotin, 0.2 µg/mL) for 1 hour at 30℃ with shaking. Then the plate was washed and incubated with 100 µl per well of SA-HRP for 0.5 hour at 30℃ with shaking. Detection was performed using an Ultra TMB Substrate for 10 minutes at room temperature in the dark. The reaction was stopped with sulfuric acid and absorbances were read on a spectrophotometer at 450 nm.

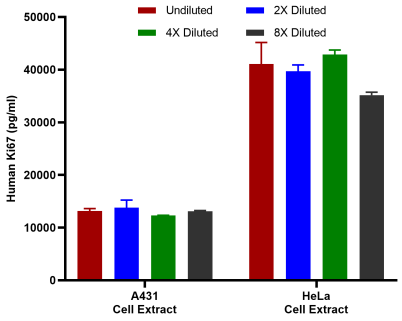

Fig2: Interpolated concentrations of native Ki67 in A431 and HeLa cell extract samples based on a 1,000 µg/mL extract load.

Capture: HA724536, Human Ki67 Rabbit mAb [PSH25-46]
Detector: HA724537, Human Ki67 Rabbit mAb [SR00-02]

The concentrations of Ki67 were measured in duplicates, interpolated from the Ki67 standard curve and corrected for sample dilution. Undiluted samples are A431 cell extract 50% and HeLa cell extract 25%. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean Ki67 concentration was determined to be 13,077 pg/mL in A431 cell extract and 39,701 pg/mL in HeLa cell extract.

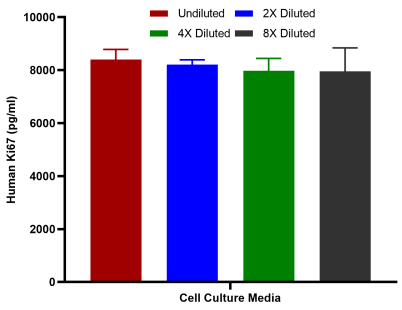

Fig3: Interpolated concentrations of spiked Ki67 in human cell culture media samples.

Capture: HA724536, Human Ki67 Rabbit mAb [PSH25-46]
Detector: HA724537, Human Ki67 Rabbit mAb [SR00-02]

The concentrations of Ki67 were measured in duplicates, interpolated from the Ki67 standard curves and corrected for sample dilution. Diluted samples are as follows: 50% cell culture media with FBS. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2).

Background References

1. Cuylen S. et al. Ki-67 acts as a biological surfactant to disperse mitotic chromosomes. Nature 535:308-312(2016).
2. Booth D.G. et al. Ki-67 is a PP1-interacting protein that organises the mitotic chromosome periphery. Elife 3:E01641-E01641(2014).