

Datasheet: HCA006

BATCH NUMBER 1608

Description:	HUMAN ANTI Ki67
Specificity:	Ki67
Format:	Purified
Product Type:	Monoclonal Antibody
Clone:	AbD02531
Isotype:	HuCAL Fab bivalent
Quantity:	0.1 mg

Product Details

Applications

This product has been reported to work in the following applications. This information is derived from testing within our laboratories, peer-reviewed publications or personal communications from the originators. Please refer to references indicated for further information. For general protocol recommendations, please visit www.bio-rad-antibodies.com/protocols.

	Yes	No	Not Determined	Suggested Dilution
Immunohistology - Frozen			▪	
Immunohistology - Paraffin			▪	
ELISA	▪			2ug/ml
Western Blotting	▪			5ug/ml
Immunofluorescence	▪			

Where this product has not been tested for use in a particular technique this does not necessarily exclude its use in such procedures. Suggested working dilutions are given as a guide only. It is recommended that the user titrates the product for use in their own system using appropriate negative/positive controls.

Target Species

Human

Species Cross Reactivity

Based on sequence similarity, is expected to react with: Rhesus Monkey, Bovine, Chimpanzee, Dog, Macaque

N.B. Antibody reactivity and working conditions may vary between species. Cross reactivity is derived from testing within our laboratories, peer-reviewed publications or personal communications from the originators. Please refer to references indicated for further information.

Product Form

Bivalent human recombinant Fab selected from the HuCAL[®] GOLD phage display library. Expressed in *E. coli* and purified using NiNTA affinity chromatography. This Fab fragment is dimerized via a helix-turn-helix motif. The antibody is tagged with a myc-tag (EQKLISEEDL) and a his-tag (HHHHHH) at the C-terminus of the antibody heavy chain --

Liquid.

Preparation	Metal chelate affinity chromatography
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Buffer Solution	Phosphate buffered saline
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Preservative Stabilisers	0.01% Thiomersal
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Approx. Protein Concentrations	Antibody concentration 0.5 mg/ml.
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Immunogen	Peptide derived from the human Ki-67 protein. Sequence GFKELFQTPG, coupled via a C-terminal cysteine to carrier proteins.
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External Database Links

UniProt:

[P46013](#) [Related reagents](#)

Entrez Gene:

[4288](#) MKI67 [Related reagents](#)

RRID AB_609758

Specificity

Human anti Ki67 antibody, clone AbD02531, recognizes the sequence GFKELFQ, which can be found eight times on the human Ki67 protein: Amino acid positions 1104-1110, 1226-1232, 1348-1354, 1590-1596, 1956-1962, 2078-2084, 2318-2324, and 2439-2445.

Ki-67 antigen is expressed in proliferating cells but not in quiescent cells. Expression of this antigen occurs preferentially during late G1, S, G2, and M phases of the cell cycle, while in cells in G0 phase the antigen cannot be detected. Consequently, Ki-67 antigen expression is used in tumor pathology to detect proliferating cells in neoplastic diseases. In cultured cells, Ki-67 is expressed in the nucleolus of interphase cells.

The Ki-67 gene contains 15 exons. The Ki-67 repeat region, within which there is a 22-amino acid Ki-67 motif, is encoded by exon 13. The shorter isoform lacks exon 7. Northern blot analysis revealed multiple transcripts ranging from approximately 8.9 to 12.5 kb in proliferating but not quiescent cells. Immunoblot analysis showed expression of 320- and 359-kDa proteins. Sequence analysis predicted that the short-lived 2,896- and 3,256-amino acid protein isoforms contain potential nuclear targeting signals, over 200 potential phosphorylation sites, 19 N-myristoylation sites, 3 amidation sites, and numerous PEST sites. Antisense oligonucleotides inhibited cellular proliferation in a dose-dependent manner, suggesting that Ki-67 protein expression may be an absolute requirement for cell proliferation. Clone AbD02531 is also known as clone 2531.

Activity

Activity was tested by indirect ELISA: The Ki-67 antigen peptide was coupled to human transferrin carrier protein. The coupled antigen (5 µg/ml) plus unrelated control proteins were immobilized on a microtiter plate. Specific binding was monitored by first adding HCA006 (2 µg/ml), then adding a secondary antibody (goat anti-human F(ab')₂ fragment

specific, AP conjugate. 1:5000 diluted). A fluorescent signal was created by adding the AP sub-strate Attophos. The signal on the antigen is at least 5-fold above background, whereas the signal on the control antigens is less than 1.5-fold above background.

Western Blotting	HCA006 detects bands of approximately 360kDa and 320kDa in Granta cell lysates
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References	<ol style="list-style-type: none">1. Jarutat, T. <i>et al.</i> (2006) Isolation and comparative characterization of Ki-67 equivalent antibodies from the HuCAL phage display library. Biol. Chem. 387: 995-1003.2. Liu, S.K. <i>et al.</i> (2011) Delta-like ligand 4-notch blockade and tumor radiation response. J Natl Cancer Inst. 103 (23): 1778-98.
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Storage	Store at +4°C or at -20°C if preferred. Storage in frost-free freezers is not recommended. This product should be stored undiluted. Avoid repeated freezing and thawing as this may denature the antibody. Should this product contain a precipitate we recommend microcentrifugation before use.
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Guarantee	12 months from date of despatch
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Acknowledgements	Sold under license of U.S. Patents 6753136, 7785859 and 8273688 and corresponding patents. This antibody was developed by Bio-Rad, Zeppelinstr. 4, 82178 Puchheim, Germany. His-tag is a registered trademark of EMD Biosciences.
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Health And Safety Information	Material Safety Datasheet documentation #10094 available at: https://www.bio-rad-antibodies.com/SDS/HCA00610094
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Licensed Use	For in vitro research purposes only, unless otherwise specified in writing by Bio-Rad.
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Regulatory	For research purposes only
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Technical Advice	Recommended protocols and further information about HuCAL recombinant antibody technology can be found in the HuCAL Antibodies Technical Manual
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Related Products

Recommended Secondary Antibodies

Mouse Anti Synthetic Peptide HISTIDINE TAG (MCA5995...) [HRP](#)

Mouse Anti Human C-MYC (MCA2200...) [Purified](#)

Goat Anti Human IgG F(ab')₂ (0500-0099...) [Alk. Phos.](#), [HRP](#)

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To find a batch/lot specific datasheet for this product, please use our online search tool at: [bio-rad-antibodies.com/datasheets](https://www.bio-rad-antibodies.com/datasheets)

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