Datasheet: HCA006

**Description:** HUMAN ANTI Ki67

**Specificity:** Ki67

**Format:** Purified

**Product Type:** Monoclonal Antibody

**Clone:** AbD02531

**Isotype:** HuCAL Fab bivalent

**Quantity:** 0.1 mg

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### 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](http://www.bio-rad-antibodies.com/protocols).

<table>
<thead>
<tr>
<th>Application</th>
<th>Yes</th>
<th>No</th>
<th>Not Determined</th>
<th>Suggested Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunohistology - Frozen</td>
<td></td>
<td></td>
<td></td>
<td>10ug/ml</td>
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<tr>
<td>Immunohistology - Paraffin (1)</td>
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<td>ELISA</td>
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<td>Western Blotting</td>
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<td>5ug/ml</td>
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<tr>
<td>Immunofluorescence</td>
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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.

(1) *This product requires antigen retrieval using heat treatment prior to staining of paraffin sections. Sodium citrate buffer pH 6.0 is recommended for this purpose.*

### 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.

### 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 (HHHHHHH) at the C-terminus of the antibody heavy chain -- Liquid.

### Preparation

Metal chelate affinity chromatography

### Buffer Solution

Phosphate buffered saline

### Preservative Stabilisers

0.01% Thiomersal
Approx. Protein Concentrations
Antibody concentration 0.5 mg/ml.

Immunogen
Peptide derived from the human Ki-67 protein. Sequence GFKELFQTPG, coupled via a C-terminal cysteine to carrier proteins.

External Database Links
UniProt: P46013 Related reagents
Entrez Gene: 4288 MKI67 Related reagents

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.

Histology Positive Control Tissue
Human tonsil.

Western Blotting
HCA006 detects bands of approximately 360kDa and 320kDa in Granta cell lysates.

References

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.

**Shelf Life**  
12 months from date of despatch.

**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.

**Health And Safety Information**  
Material Safety Datasheet documentation #10094 available at:  

**Licensed Use**  
For in vitro research purposes only, unless otherwise specified in writing by Bio-Rad.

**Regulatory**  
For research purposes only

**Technical Advice**  
Recommended protocols and further information about HuCAL recombinant antibody technology can be found in the HuCAL Antibodies Technical Manual

**Related Products**

**Recommended Secondary Antibodies**

- Mouse Anti Synthetic Peptide HISTIDINE TAG (MCA5995...) **HRP**
- Goat Anti Human IgG F(ab')2 (0500-0099...) **Alk. Phos., HRP**
- Mouse Anti Human C-MYC (MCA2200...) **Purified**

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