

## Ki67

### Immunohistochemistry

<https://www.abcam.com/protocols/immunostaining-paraffin-frozen-free-floating-protocol>

#### Target Overview in IHC Application

Expression of Ki67 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 (at protein level). (PMID:6206131)

#### Here are a few tips to help ensure the best results in IHC:

<b>Sample Fixation</b>	<ul style="list-style-type: none"> <li>The ideal fixation time will depend on the size of the tissue block and the type of tissue, but <b>fixation between 18–24h</b> is suitable for most samples.</li> </ul>
	<ul style="list-style-type: none"> <li>Under-fixation can lead to edge staining, with strong signal on the edges of the section and no signal in the middle.</li> </ul>
	<ul style="list-style-type: none"> <li>Over-fixation can mask the epitope; antigen retrieval can help overcome this masking, but if the tissue has been fixed for a long period of time (i.e. over a weekend), there may be no signal even after antigen retrieval.</li> </ul>

#### You should pay attention to these notes to maximize the signal:

- ✓ **Antigen retrieval:** Heat in citrate buffer pH 6 for 20-30 minutes or enzymatic (trypsin, proteinase K). (Necessary if fixed in PFA)
- ✓ **Permeabilize the tissues:** 0.2% Triton in PBS for 10 minutes

### Immunocytochemistry/Immunofluorescence

<https://www.abcam.com/protocols/immunocytochemistry-immunofluorescence-protocol>

#### Target Overview in ICC/IF Application

Ki67 locates in chromosomes and nucleus. Therefore, **4% PFA fixation** is recommended. And **permeabilize the cells** (0.1% TritonX-100 in PBS for 5 minutes) in ICC assay is essential.

#### Here are a few tips to help ensure the best results in ICC/IF:

<b>Sample Fixation Permeabilization</b>	<ul style="list-style-type: none"> <li>For nuclear proteins, fix cells in <b>4% PFA</b> (20 minutes, room temperature) is recommended.</li> </ul>
	<ul style="list-style-type: none"> <li>Please <b>do not over-fix</b> your samples, which will reduce signal.</li> </ul>
	<ul style="list-style-type: none"> <li>It is recommended to incubate cells with <b>0.1% Triton-X for 5 min</b> to detect nuclear antigen.</li> </ul>

<b>Protein Function</b>	<p>Ki67 is required to maintain individual mitotic chromosomes dispersed in the cytoplasm (PMID: 27362226). It associates with the surface of the mitotic chromosome, the perichromosomal layer, and covers a substantial fraction of the chromosome surface (PMID: 27362226). Ki67 prevents chromosomes from collapsing into a single chromatin mass by forming a steric and electrostatic charge barrier: the protein has a high net electrical charge and acts as a surfactant, dispersing chromosomes and enabling independent chromosome motility (PMID: 27362226). Binds DNA, with a preference for supercoiled DNA and AT-rich DNA (PMID: 10878551). It may play a role in chromatin organization (PMID: 24867636).</p> <p style="text-align: right;">SwissProt: P46013</p>				
<b>Expression</b>	<p>Expression 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 (at protein level).  <span style="float: right;">PMID: 6206131</span></p> <p>Present at <b>highest level in G2 phase</b> and <b>during mitosis</b> (at protein level). In interphase, forms fiber-like structures in fibrillarin-deficient regions surrounding nucleoli.  <span style="float: right;">PMID: 2674163 / PMID: 8799815</span></p>				
<b>Location</b>	Chromosome. Nucleus				
<b>Isoforms</b>	<p>Human Isoform 1: 319 kD (predicted)          Isoform 2: 359 kD (predicted)</p> <p>Mouse Isoform 1: 325 kD (predicted)          Isoform 2: 351 kD (predicted)</p> <p>Rat Isoform 1: 343 kD (predicted)</p> <p><b>The observed band size of Ki67 may not be the same as predicted MWs in WB due to the different forms of Ki67.</b></p>				
<b>Modifications</b>	<p>Phosphorylation/Hyperphosphorylation in mitosis.          Hyperphosphorylated form does not bind DNA.  <span style="float: right;">PMID: 10502411 / PMID: 10653604</span></p> <p><b>The observed band size of Ki67 may not be the same as predicted MWs in WB due to these modifications.</b></p>				
<b>Positive Controls</b>	<table border="0" style="width: 100%;"> <tr> <td style="width: 50px;"><b>IHC</b></td> <td>Human tonsil tissue            Mouse tumour tissue, Mouse embryonic skin tissue            Rat esophagus, small intestine and liver tissue.</td> </tr> <tr> <td><b>ICC</b></td> <td>HeLa and HAP1 cells.            Rat cardiomyocytes</td> </tr> </table>	<b>IHC</b>	Human tonsil tissue Mouse tumour tissue, Mouse embryonic skin tissue Rat esophagus, small intestine and liver tissue.	<b>ICC</b>	HeLa and HAP1 cells. Rat cardiomyocytes
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