**Product datasheet**

**Anti-Rhodopsin antibody [1D4] ab5417**

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### Overview

**Product name**  Anti-Rhodopsin antibody [1D4]  
**Description**  Mouse monoclonal [1D4] to Rhodopsin  
**Host species**  Mouse  
**Specificity**  ab5417 detects Rhodopsin from human and bovine retinal samples. Data from Yin J et al., 2012 (PMID 22743318) indicates that in Zebrafish ab5417 appears to recognize Red Opsin rather than Rhodopsin.  
**Tested applications**  Suitable for: IHC-FoFr, IHC-Fr, ELISA, ICC, IP, WB, IHC-P  
**Species reactivity**  Reacts with: Mouse, Rat, Cow, Human, Zebrafish, Amphibian  
**Predicted to work with:** Rabbit  
**Immunogen**  Other Immunogen Type corresponding to Bovine Rhodopsin.  
**Epitope**  The epitope for this antibody has been localized to the C-terminal nine amino acids of bovine rhodopsin known as the 1D4 epitope.

### Properties

**Form**  Liquid  
**Storage instructions**  Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle.  
**Storage buffer**  Preservative: 0.05% Sodium azide  
Constituents: 99% PBS, 0.1% BSA  
**Purity**  Protein G purified  
**Primary antibody notes**  Vision involves the conversion of light into electrochemical signals that are processed by the retina and subsequently sent to and interpreted by the brain. The process of converting light to an electrochemical signal begins when the membrane-bound protein, rhodopsin, absorbs light within the retina. Photoexcitation of rhodopsin causes the cytoplasmic surface of the protein to become catalytically active. In the active state, rhodopsin activates transducin, a GTP binding protein. Once activated, transducin promotes the hydrolysis of cGMP by phosphodiesterase (PDE). The decrease of intracellular cGMP concentrations causes the ion channels within the outer segment of the rod or cone to close, thus causing membrane hyperpolarization and, eventually, signal transmission. Rhodopsin’s activity is believed to be shut off by its phosphorylation followed by binding of the soluble protein arrestin.
Clonality: Monoclonal
Clone number: 1D4
Isotype: IgG1

Applications

Our Abpromise guarantee covers the use of ab5417 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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<tbody>
<tr>
<td>IHC-FoFr</td>
<td></td>
<td>1/1000. PubMed: 19587120</td>
</tr>
<tr>
<td>IHC-Fr</td>
<td>★★★★★</td>
<td>Use at an assay dependent concentration. PubMed: 22743318</td>
</tr>
<tr>
<td>ELISA</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>ICC</td>
<td></td>
<td>1/1000.</td>
</tr>
<tr>
<td>IP</td>
<td></td>
<td>Use at an assay dependent concentration. Use at an assay dependent dilution.</td>
</tr>
<tr>
<td>WB</td>
<td>★★★★☆☆</td>
<td>1/1000. Detects a band of approximately 40 kDa.</td>
</tr>
<tr>
<td>IHC-P</td>
<td></td>
<td>1/100 - 1/1000.</td>
</tr>
</tbody>
</table>

Target

Function: Photoreceptor required for image-forming vision at low light intensity. Required for photoreceptor cell viability after birth. Light-induced isomerization of 11-cis to all-trans retinal triggers a conformational change leading to G-protein activation and release of all-trans retinal.

Tissue specificity: Rod shaped photoreceptor cells which mediates vision in dim light.

Involvement in disease: Retinitis pigmentosa 4
Night blindness, congenital stationary, autosomal dominant 1

Sequence similarities: Belongs to the G-protein coupled receptor 1 family. Opsin subfamily.

Post-translational modifications: Phosphorylated on some or all of the serine and threonine residues present in the C-terminal region.
Contains one covalently linked retinal chromophore.

Cellular localization: Membrane. Synthesized in the inner segment (IS) of rod photoreceptor cells before vectorial transport to the rod outer segment (OS) photosensory cilia.

Images
Deletion of Arf4 from the retina does not disrupt rhodopsin localization or photoreceptor morphology.

E. Arf4 immunostaining in Arf4\textsuperscript{floox}/CagCreER experimental and control retinal cross-sections. Image of the photoreceptor IS where the biosynthetic membranes are localized. Eyes were collected at P34. Scale bar = 10 μm.

F. Rhodopsin immunostaining in Arf4\textsuperscript{floox}/CagCreER experimental and control retinal cross-sections. Eyes were collected at P34. Scale bar = 10 μm.

G. Comparative analysis of photoreceptor morphology in Arf4\textsuperscript{floox}/CagCreER experimental and control retinal cross-sections. Eyes were collected at P41. Scale bar = 20 μm.

OS = outer segment, IS = inner segment, ONL = outer nuclear layer, OPL = outer plexiform layer.

Immunohistochemical analysis of formalin-fixed mouse retinal tissue, labeling rhodopsin with ab5417 at a 1:50 dilution in 3% BSA-PBS solution and incubated at 4°C overnight in a high humidity environment.

A DyLight\textsuperscript{®} 488 secondary antibody was used (green) incubated at room temperature in the dark. The tissue was counterstained with DAPI against DNA, showing nuclear compartments. Prior to staining the formalin-fixed tissue was permeabilized with 0.1% Triton X-100 in TBS for between 5 and 10 minutes, then blocked with 3% BSA-PBS for 30 minutes at room temperature.

The left image is a negative control with only the secondary antibody and the right image is in the presence of ab5417 and the secondary.
Immunohistochemical analysis of paraffin-embedded mouse retinal tissue labeling Rhodopsin with ab5417.

Secondary used was HRP conjugated. Prior preparation was initiated by antigen retrieval using 10mM sodium citrate at pH 6.0, then the sample was microwaved for 8 to 15 minutes. Subsequent to retrieval the retinal tissue was blocked for 15 minutes at room temperature with 3% hydrogen peroxide. The sample was then incubated with ab5417 in 3% BSA-PBS at 4°C at a dilution of 1:1000, overnight. Hematoxylin was used to counterstain the tissue. The left side of the image is shown as a negative control and is the tissue in the absence of ab5417, the right side is in the presence of the counterstain, ab5417 and the HRP conjugated secondary.

Immunohistochemistry (Frozen sections) image of anti-Rhodopsin antibody (ab5417) staining on a Zebrafish eye.

This tissue section was stained with anti-Rhodopsin antibody (green) and Red Opsin antibody (red) for 16 hours at 4°C. Nuclei were stained with DAPI (blue). This image suggests that ab5417 detects Red Opsin and not Rhodopsin in the Zebrafish. For more information please see Yin J. et al., 2012.

Immunohistochemical analysis of formalin-fixed human retinal tissue, labeling rhodopsin with ab5417 at a 1:50 dilution in 3% BSA-PBS solution and incubated at 4°C overnight in a high humidity environment.

A DyLight® 488 secondary antibody was used (green) incubated at room temperature in the dark. The tissue was counterstained with DAPI against DNA, showing nuclear compartments. Prior to staining the formalin-fixed tissue was permeabilized with 0.1% Triton X-100 in TBS for between 5 and 10 minutes, then blocked with 3% BSA-PBS for 30 minutes at room temperature. The left image is a negative control with only the secondary antibody and the right image is in the presence of ab5417 and the secondary.
Anti-Rhodopsin antibody [1D4] (ab5417) at 1/500 dilution + HL60 (Human promyelocytic leukemia cell line) cell lysate at 25 µg

**Observed band size:** 40 kDa

*why is the actual band size different from the predicted?*

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**Please note:** All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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