# ANTIBODIES ONLINE

# Datasheet for ABIN7271760 anti-RGR antibody

1 Validation

1 Publication



#### Overview

Quantity:	500 µg
Target:	RGR
Reactivity:	Mouse
Host:	Rabbit
Clonality:	Polyclonal
Conjugate:	This RGR antibody is un-conjugated
Application:	Western Blotting (WB), Immunohistochemistry (IHC)
Product Details	
Immunogen:	A synthetic peptide from mouse retinal G protein coupled receptor (RGR) conjugated to blue
	carrier protein was used as the antigen. Available under product number ABIN7271761.
Specificity:	Specific for RGR.
Cross-Reactivity (Details):	Mouse, marmoset. Other species not yet tested.
Purification:	IgG fraction

### Target Details

Target:	RGR
Abstract:	RGR Products
Background:	FUNCTION: Receptor for all-trans- and 11-cis-retinal. Binds preferentially to the former and may catalyze the isomerization of the chromophore by a retinochrome-like mechanism. SUBCELLULAR LOCATION: Membrane; Multi-pass membrane protein.

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Target Details	
UniProt:	Q9Z2B3
Application Details	
Application Notes:	A concentration of 10-50 ug/ml is recommended. The optimal concentration should be determined by the end user. Not yet tested in other applications.
Comment:	This antibody is also available as whole serum under the product number ABIN350792. The immunogenic peptide that can be used as blocking peptide is available under the number ABIN7271761.
Restrictions:	For Research Use only
Handling	
Format:	Lyophilized
Reconstitution:	Reconstitute in 500 ul of sterile water. Centrifuge to remove any insoluble material.
Storage:	4 °C,-20 °C
Storage Comment:	Maintain the lyophilised/reconstituted antibodies frozen at -20°C for long term storage and refrigerated at 2-8°C for a shorter term. When reconstituting, glycerol (1:1) may be added for an additional stability. Avoid freeze and thaw cycles.
Expiry Date:	12 months
Publications	
Product cited in:	Tworak, Kolesnikov, Hong, Choi, Luu, Palczewska, Dong, Lewandowski, Brooks, Campello, Swaroop, Kiser, Kefalov, Palczewski: "Rapid RGR-dependent visual pigment recycling is mediated by the RPE and specialized Müller glia." in: <b>Cell reports</b> , Vol. 42, Issue 8, pp. 112982, (

2023) (PubMed).





#### Successfully validated (Immunohistochemistry (IHC))

by Palczewski Lab, Center For Translational Vision Research, UC Irvine Report Number: 104459 Date: Sep 21 2022

Target:	RGR
Lot Number:	Rb3134
Method validated:	Immunohistochemistry (IHC)
Positive Control:	Retina cryosection from Rgr <sup>+/+</sup> mouse, animal validated by genotyping
Negative Control:	Retina cryosection from Rgr <sup>-/-</sup> mouse, animal validated by genotyping
Notes:	Passed. Presence of specific signal in the RPE cell layer in $Rgr^{+/+}$ section, and its absence in a
	respective Rgr <sup>-/-</sup> section was considered as indication of ABIN7271760 being immunoreactive
	to Rgr.
Primary Antibody:	ABIN7271760
Secondary Antibody:	donkey anti-rabbit AF488-conjugated antibody (Thermo Fisher Scientific, A-21206)
Protocol:	Collect eyes from mice and fix with paraformaldehyde 4% (Electron Microscopy Sciences,
	15710) in 1x PBS for 30 min at RT.
	Cryoprotection with sucrose series:
	<ul> <li>Wash in 10% sucrose in 1x PBS.</li> </ul>
	<ul> <li>Immerse in 10% sucrose in 1x PBS for 30 min at RT.</li> </ul>
	<ul> <li>Wash in 20% sucrose in 1x PBS.</li> </ul>
	<ul> <li>Immerse in 20% sucrose in 1x PBS for 30 min RT.</li> </ul>
	<ul> <li>Wash in 30% sucrose in 1x PBS.</li> </ul>
	<ul> <li>30% sucrose overnight at 4°C.</li> </ul>
	Embed eyes in OCT compound (Tissue-Tek O.C.T. Compound, 4583).
	<ul> <li>Cut retinal sections at a thickness of 12 µm on a cryostat.</li> </ul>
	<ul> <li>Air dry sections for 15 min at RT, store at -80°C until use.</li> </ul>
	<ul> <li>Sections brought to room temp, rehydrated in 1x PBS for 1h.</li> </ul>
	Incubate sections in blocking buffer (1x PBS, 3% BSA (Sigma-Aldrich, A7030), 3% Donkey
	serum (Sigma-Aldrich, S30-100ML), 0.1% Triton X-100 (Sigma-Aldrich, X100-500ML)) for 1 l at RT.
	Incubate sections with primary rabbit anti-Rgr antibody (antibodies-online, ABIN7271760, lo
	Rb3134) diluted 1:100 in blocking buffer ON at RT. Include a no primary antibody negative controls.
	<ul> <li>Rinse sections 3 times with 1x PBS, 0.1% Triton X-100. Keep negative controls in a separate</li> </ul>

	<ul> <li>container.</li> <li>Incubate sections with secondary donkey anti-rabbit AF488 -conjugated antibody (Thermo Fisher Scientific, A-21206) diluted 1:500 in blocking buffer for 1 h at RT.</li> <li>Rinse sections with once PBS, 0.1% Triton X-100 for 5 min at RT.</li> <li>Incubate sections in DAPI (Thermo Fisher Scientific, 62248) in 1x PBS, 0.1% Triton X-100 for 15 min.</li> <li>Rinse sections 3 times with 1x PBS, 0.1% Triton X-100 for 5 min at RT.</li> <li>Mount sections in VECTASHIELD® HardSet™ Antifade Mounting Medium (Vector Laboratories, H-1400) mounting medium.</li> <li>Acquire images with a fluorescence microscope and appropriate filter settings. For the validation purposes Keyence BZ-X800E fluorescence microscope was used with following filters: BZ-X DAPI for DAPI, BZ-X GFP for AF488, BZ-X Cy5 for Cy5. Images were taken at 100x magnification.</li> </ul>
Experimental Notes:	<ul> <li>The experiments involved comparison of seven different anti-mouse Rgr antisera produced in rabbit. Anti-Rgr-antiserum Rb3134-24117-WS (ABIN7271760) proved to be immunoreactive and showed much lower background level.</li> <li>Rgr in mouse eye is predominantly expressed in the RPE cell layer, and to much lesser extent in the Muller glia (for details see Fig 10, PMID31694912).</li> <li>To aid orientation in the tissue layers PNA (cone photoreceptor) counterstain was included in the staining (Vector Labs, PNA-Cy5 CL-1075-1) together with the secondary Ab incubation, at 1:500 diulution.</li> </ul>

#### Image for Validation report #104459



## Validation image no. 1 for anti-Retinal G Protein Coupled Receptor (RGR) antibody (ABIN7271760)

Retinal sections from the Rgr<sup>+/+</sup> and Rgr<sup>-/-</sup> animals immunostained with ABIN7271760. DAPI staining shows localization of the inner (INL) and outer (ONL) nuclear layer of the mouse retina. PNA staining was used to visualize cone inner and outer segments. RPE rests above cones, and is the site of highest Rgr expression in mouse eye. Magnification 100x.

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