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Datasheet for ABIN7254061 anti-RDH5 antibody

Validation

1 Image

Publication



Overview

1

Quantity:	200 µL
Target:	RDH5
Reactivity:	Human, Mouse
Host:	Rabbit
Clonality:	Polyclonal
Conjugate:	This RDH5 antibody is un-conjugated
Application:	ELISA, Immunohistochemistry (IHC)

Product Details

Immunogen:	Fusion protein of human RDH5
Isotype:	lgG
Characteristics:	Polyclonal Antibody
Purification:	Antigen affinity purification

Target Details

Target:	RDH5
Alternative Name:	RDH5 (RDH5 Products)
Background:	This gene encodes an enzyme belonging to the short-chain dehydrogenases/reductases (SDR) family. This retinol dehydrogenase functions to catalyze the final step in the biosynthesis of 11-
	cis retinaldehyde, which is the universal chromophore of visual pigments. Mutations in this
	gene cause autosomal recessive fundus albipunctatus, a rare form of night blindness that is

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Target Details		
	characterized by a delay in the regeneration of cone and rod photopigments. Alternative splicing results in multiple transcript variants. Read-through transcription also exists between this gene and the neighboring upstream BLOC1S1 (biogenesis of lysosomal organelles complex-1, subunit 1) gene.	
UniProt:	Q92781	
Application Details		
Application Notes:	IHC 1:50-1:100, ELISA 1:5000-1:10000	
Restrictions:	For Research Use only	
Handling		
Format:	Liquid	
Concentration:	0.84 mg/mL	
Buffer:	PBS with 0.05 % Sodium azide and 40 % Glycerol, pH 7.4	
Preservative:	Sodium azide	
Precaution of Use:	This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.	
Storage:	-20 °C	
Storage Comment:	Store at -20°C. Avoid freeze / thaw cycles.	
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: Cell reports , Vol. 42, Issue 8, pp. 112982, (2023) (PubMed).	



Immunohistochemistry (Paraffin-embedded Sections)

Image 1. Immunohistochemistry of paraffin-embedded Human brain tissue using RDH5 Polyclonal Antibody at dilution of 1:50(x200)

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Successfully validated (Immunohistochemistry (IHC))

by Palczewski Lab, Center For Translational Vision Research, UC Irvine Report Number: 104468 Date: Mar 23 2023

Farget:	RDH5
ot Number:	SG5433
Method validated:	Immunohistochemistry (IHC)
Positive Control:	Retina cryosection from B6 Albino (B6(Cg)-Tyrc-2J/J) animal
Negative Control:	Retina cryosection from B6 Albino (B6(Cg)-Tyrc-2J/J) animal
	No primary antibody
Notes:	Passed. Presence of specific signal in the RPE cell layer was considered as indication of
	specific immunoreactivity using the anti-RDH5 antibody ABIN7254060.
Primary Antibody:	ABIN7254060
Secondary Antibody:	donkey anti-rabbit AF647-conjugated antibody (Abcam, 150075)
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:
	 Wash in 10% sucrose in 1x PBS.
	 Immerse in 10% sucrose in 1x PBS for 30 min at RT.
	 Wash in 20% sucrose in 1x PBS.
	 Immerse in 20% sucrose in 1x PBS for 30 min RT.
	 Wash in 30% sucrose in 1x PBS.
	○ 30% sucrose ON at 4°C.
	Embed eyes in OCT compound (Tissue-Tek O.C.T. Compound, 4583).
	 Cut retinal sections at a thickness of 12 µm on a cryostat.
	 Air dry sections for 15 min at RT, store at -80°C until use.
	Bring sections to RT and rehydrate in 1x PBS for 1 h.
	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 H
	at RT.
	Incubate sections with primary rabbit anti-RDH5 antibody (antibodies-online, ABIN7254060
	lot SG5433) diluted 1:50 in blocking buffer ON at RT. Include a no primary antibody negative
	controls. Additionally, counterstaing with primary mouse anti-CRALBP antibody (Thermo Fisher Scientific, MA1-813).

	 Rinse sections 3 times with 1x PBS, 0.1% Triton X100. Keep negative controls in a separate container. Incubate sections with secondary AF647-conjugated donkey anti-rabbit antibody (Abcam, Ab150075) or AF488-conjugated donkey anti-mouse antibody (Thermo Fisher Scientific, A32766) diluted 1:500 in blocking buffer for 1 h at RT. Rinse sections once with 1x PBS, 0.1% Triton X-100 for 5 min at RT. Incubate sections in 1x DAPI (Thermo Fisher Scientific, 62248) in 1x PBS, 0.1% Triton X-100 for 15 min at RT. Rinse sections 3x with 1x PBS, 0.1% Triton X-100 for 5 min at RT. Rinse sections in VECTASHIELD® HardSet[™] Antifade Mounting Medium (Vector Laboratories, H-1400) mounting medium. 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 AF647. Images were taken at 10x and 40x magnification.
Experimental Notes:	 Experiment involved validation of the specificity of 4 antibodies against different Rdh proteins: Rdh5 (ABIN7254060), Rdh10 (ABIN7118460), Rdh11 (ABIN966957), and Rdh12 (ABIN7167836). All 4 proteins are important for eye function and highly expressed in neural retina and/or RPE. Validation is based on comparison of each staining with known pattern of expression in the mouse retina. For review highlighting each Rdh localization see PMID20801113. To aid orientation in the cell layers anti-Cralbp counterstain was included in the staining (Thermo MA1-813). Cralbp (Rlbp1) is highly expressed in RPE and Müller glia cells in mouse retina.

Image for Validation report #104468



Validation image no. 1 for anti-Retinol Dehydrogenase 5 (11-Cis/9-Cis) (RDH5) antibody (ABIN7254060)

Retinal sections from the wild-type (B6 albino) mice immunostained with anti-RDH5 antibody ABIN7254060. DAPI staining shows localization of the inner (INL) and outer (ONL) nuclear layer of the mouse retina. Cralbp (Rlbp1) costaining was used to visualize RPE and Müller glia cells in the retina. Presence of specific signal in the RPE cell layer confirms specific immunoreactivity.

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