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## anti-CLNS1A antibody



Validation



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Quantity:	100 μL	
Target:	CLNS1A	
Reactivity:	Human	
Host:	Rabbit	
Clonality:	Polyclonal	
Conjugate:	This CLNS1A antibody is un-conjugated	
Application:	Western Blotting (WB), Flow Cytometry (FACS), Immunohistochemistry (IHC)	

#### **Product Details**

Immunogen:	A synthetic Peptide of human CLNS1A	
Isotype:	IgG	
Purification:	Affinity purification	

### **Target Details**

Target:	CLNS1A
Alternative Name:	CLNS1A (CLNS1A Products)
Background:	This gene encodes a protein that functions in multiple regulatory pathways. The encoded
	protein complexes with numerous cytosolic proteins and performs diverse functions including
	regulation of small nuclear ribonucleoprotein biosynthesis, platelet activation and cytoskeletal
	organization. The protein is also found associated with the plasma membrane where it
	functions as a chloride current regulator. Pseudogenes of this gene are found on chromosomes

#### **Target Details**

Target Details	
	1, 4 and 6. Several transcript variants encoding different isoforms have been found for this
	gene.
Molecular Weight:	26.215 kDa
Gene ID:	1207
UniProt:	P54105
Pathways:	Ribonucleoprotein Complex Subunit Organization
Application Details	
Application Notes:	WB 1:500 - 1:1000
	IHC 1:20 - 1:50
	FC 1:20 - 1:50
Restrictions:	For Research Use only
Handling	
Format:	Liquid
Buffer:	PBS with 0.02 % sodium azide, 50 % glycerol, pH 7.3.
Preservative:	Sodium azide
Precaution of Use:	This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which
	should be handled by trained staff only.
Handling Advice:	Avoid repeated freeze/thaw cycles.
Storage:	-20 °C
Storage Comment:	Store at -20C. Avoid freeze / thaw cycles.





#### Successfully validated (Western Blotting (WB))

by AG Gruss, Institut für Genetik, Rheinische Friedrich-Wilhelms-Universität Bonn

Report Number: 102909

Date: Sep 20 2018

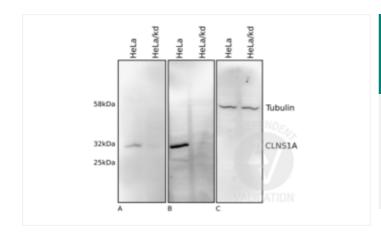
Target:	CLNS1A
Lot Number:	03R181011
Method validated:	Western Blotting (WB)
Positive Control:	HeLa cells Independent antibody
Negative Control:	HeLa cells, CLNS1A knockdown using Dharmacon smartpool siRNA
Notes:	Passed. ABIN4903315 specifically reveals endogenous CLNS1A in HeLa cell lysates while no signal is detected subsequently to knock-down of the antigen.
Primary Antibody:	ABIN4903315
Secondary Antibody:	HRP-conjugated goat anti-rabbit IgG (H+L) (Jackson ImmunoResearch, 111-035-003)
Protocol:	<ul> <li>Seed 3x10<sup>4</sup> HeLa cells in DMEM (PAN Biotech, P04-41450, 2730618) supplemented with 10% FBS (PAN Biotech, P03-3302), without antibiotics, in 6 well plates in 2ml media.</li> <li>Grow cells for 24h at 37°C and 5% CO<sub>2</sub> to 70% confluency.</li> <li>Change medium.</li> <li>Transfect cells with 60pmol ON-TARGETplus SmartPool siRNA human (Dharmacon, L-012571-00-0005, lot 170831) in 125µl 150mM NaCl added to 7.5µl Lipofectamine RNAiMax (13778030, lot 1911816) in 125µl 150mM NaCl.</li> <li>Grow cells for 24h at 37°C and 5% CO<sub>2</sub>.</li> <li>Change medium.</li> <li>Grow cells for 72h at 37°C and 5% CO<sub>2</sub>.</li> <li>Wash cells once with PBS (PAN Biotech, P04-36500, lot 3620618).</li> <li>Detach cells with 100µl trypsin per well.</li> <li>For WB the cells were once washed with PBS (PAN Biotech) before treated with 100µl of Trypsin (PAN Biotech, P10-023100, lot 6860417) per well.</li> <li>Once cells are detached add 900µl medium.</li> <li>Count cells.</li> <li>Add 40µl 1x Laemmli buffer (Roth, K929.1, lot 227259243) to 2x10<sup>5</sup> cells. Heat up to 99°C for 10min.</li> <li>Separate 10µl of the lysates on a freshly cast denaturing 12% SDS-PAGE gel in an electrophoresis chamber for 1h at 120V.</li> </ul>

- Transfer proteins onto PVDF membrane (Merck, IPVH0010, lot K5KN7128I) with a wet blotting system for 1h at 80V.
- Block the membrane with 1x Roti Block (Roth, A151.2, lot 148270222) for 30min at RT.
- · Incubation with primary
  - o rabbit anti-CLNS1A antibody (antibodies-online, ABIN4903315, lot 03R181011) diluted 1:500 in 1x Roti Block for 1h at RT,
  - o mouse anti-CLNS1A antibody (Santa Cruz, sc-271454, G3010) diluted 1:3000 in 1x Roti Block for 1h at RT, or
  - orat anti-tubulin antibody (YOL 1/34 gifted) diluted 1:1000 in 1x Roti Block for 1h at RT.
- Wash membrane 2x for 10min with 1x TBST.
- · Incubation with secondary
  - HRP-conjugated goat anti-rabbit IgG (H+L) (Jackson ImmunoResearch, 111-035-003) diluted 1:1000 in 1x Roti Block for 1h at RT,
  - o goat anti-mouse IgG (H+L) (Jackson ImmunoResearch, 115-005-003, lot 128525) diluted 1:10000 in PBS for 45min at RT, or
  - HRP-conjugated goat anti-rat IgG (H+L) (Jackson ImmunoResearch, 112-035-003) diluted 1:10000 in PBS for 45min at RT.
- Wash membrane 5x for 5 min with TBS-T.
- Reveal protein bands using ECL (GE Healthcare, RPN3244V2, 9633722) on an LAS mini system (GE healthcare).

**Experimental Notes:** 

Western blot using ABIN933127 on HeLa cell extracts reveals a band of the expected molecular weight. The same band is also detected by an independent CLNS1A antibody and disappears upon siRNA knock-down.

#### Image for Validation report #102909



Validation image no. 1 for anti-Chloride Channel, **Nucleotide-Sensitive**, (CLNS1A) **1A** antibody (ABIN4903315)

Western blot analysis of HeLa cell lysates without (HeLa) or with siRNA knock-down (HeLa/kd). CLNS1A was specifically detected using ABIN4903315 (A) and an independent antibody (B). Tubulin serves as loading control (C).