Datasheet for ABIN7298962

anti-INPP5E antibody (C-Term)

1 Validation

1 Image

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Overview

Quantity:	100 µL
Target:	INPP5E
Binding Specificity:	C-Term
Reactivity:	Human, Mouse, Rat
Host:	Rabbit
Clonality:	Polyclonal
Conjugate:	This INPP5E antibody is un-conjugated
Application:	Western Blotting (WB)

Product Details

Immunogen:	KLH-conjugated synthetic peptide encompassing a sequence within the C-term region of human INPP5E.
Specificity:	Recognizes endogenous levels of INPP5E protein.
Characteristics:	Rabbit polyclonal antibody to INPP5E
Purification:	The antibody was purified by immunogen affinity chromatography.

Target Details

Target:	INPP5E
Alternative Name:	INPP5E (INPP5E Products)
Background:	72 kDa inositol polyphosphate 5-phosphatase, Phosphatidylinositol 4,5-bisphosphate 5-

Target Details

	phosphatase, Phosphatidylinositol polyphosphate 5-phosphatase type IV
Gene ID:	56623, 64436
UniProt:	Q9NRR6, Q9JII1, Q9WVR1

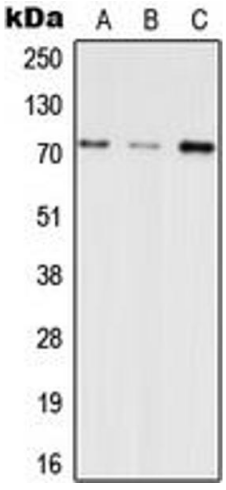
Application Details

Application Notes:	WB (1:500 - 1:1000)
Restrictions:	For Research Use only

Handling

Format:	Liquid
Buffer:	Liquid in 0.42 % Potassium phosphate, 0.87 % Sodium chloride, pH 7.3, 30 % glycerol, and 0.01 % sodium azide.
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:	Shipped at 4°C. Upon delivery aliquot and store at -20°C for one year. Avoid freeze/thaw cycles.
Expiry Date:	12 months

Images



Western Blotting

Image 1. Western blot analysis of INPP5E expression in HEK293T (A), SP2/0 (B), H9C2 (C) whole cell lysates.

Validation report #101129 for Western Blotting (WB)

Successfully validated (Western Blotting (WB))

by Children's Hospital of Eastern Ontario Research Institute

Report Number: 101129

Date: Nov 10 2017

Target:	INPP5E
Lot Number:	CP1D10A
Method validated:	Western Blotting (WB)
Positive Control:	Inpp5e$^{+/+}$ 4T1 mouse breast carcinoma cell line (transfected with non-targeting Cas9 gRNA), treated with an inducer (unpublished)
Negative Control:	Inpp5e^{em} 4T1 cell line (CRISPR/Cas9 endonuclease mediated Inpp5e knock-out)
Notes:	Passed. ABIN2704925 specifically recognizes Inpp5e in murine Inpp5e$^{+/+}$ 4T1 cells upon induction aside from other unspecific bands.
Primary Antibody:	ABIN2704925
Secondary Antibody:	goat anti-rabbit IgG (H+L)-HRP-conjugated antibody (BioRad, 172-1019, lot 350001898)
Protocol:	• Grow Inpp5e$^{+/+}$ and Inpp5e^{em} 4T1 murine cells in HyClone TM RPMI-1640 medium (GE Healthcare Life Science, SH30027.01, lot AC11016269) supplemented with fetal bovine serum (Sigma, F1051-500ML, lot 17D192) and HyClone penicillin – streptomycine solution (GE Healthcare Life Science, SV30010, lot SH40003.01), at 37°C and 5% CO_2 in 3ml on a 6-well plate to 95% confluency. • Treat cells with an "inducer" (unpublished) to increase Inpp5e expression. • Lyse cells in 200µl per well of cold RIPA buffer (150mM NaCl, 1.0% IGEPAL CA-630 Sigma, 0.5% sodium deoxycholate, 0.1% SDS, 50mM Tris, pH8.0). • Determine the total protein content of the lysates using DC Protein Assay (BioRad, 500-0114, lot 200008008). • Denature 30µg of total protein for 5min at 95°C in 5x Laemmli SDS sample buffer and subsequently separate proteins on a non-/denaturing 10% SDS-polyacrylamide gel in a mini-Protean electrophoresis cell (BioRad) for 90min at 100V. • Transfer proteins onto 0.2µm nitrocellulose membrane (BioRad, 1620112, lot A10088580) with a MiniTransblot Cell (BioRad, 1703930) system for 60V at 300mA. • Block the membrane with TBS containing 0.1% Tween 20 (TBST) and 5% skim milk for 30min at RT. • Incubation with primary rabbit anti-INNP5E antibody (antibodies-online, ABIN2704925, CP1D10A) diluted 1:1000 in TBST containing 5% BSA and 0.05% NaN_3 ON at 4°C. • Wash membrane 3x with TBST.

Validation report #101129 for Western Blotting (WB)

- Incubation with secondary goat anti-rabbit IgG (H+L)-HRP-conjugated antibody (BioRad, 172-1019, lot 350001898) diluted 1:5000 in TBST for 1h at RT.
- Wash membrane 5x with TBST.
- Reveal protein bands using Clarity Western ECL Substrate (BioRad, 1705060, lot 102030837) on a ChemiDoc Imaging System (Biorad, 17001401).

Experimental Notes:

- ABIN2704925 reveals a single band in the "wild type" cell line with treatment. This protein band is abrogated in the CRISPR/Cas9 targeted cells and under non-inducing conditions, thus demonstrating immunospecificity of ABIN2704925 for Inpp5e in the mouse.
- We observed a lower molecular weight than the predicted size of 72kDa for Inpp5e. We suspect that this might be a splice isoform. We observed a similar band also with a different anti-INPP5E antibody (Thermo Fisher Scientific, PA5-37119) using similar experiment condition.
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- ABIN2704925 also recognizes potentially unspecific bands at various sizes.
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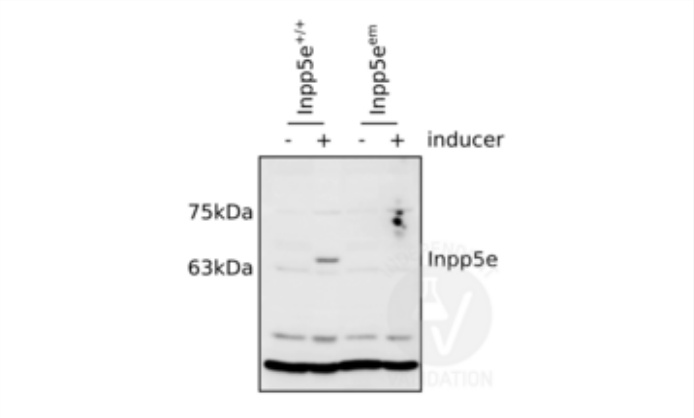
Image for Validation report #101129



Validation image no. 1 for anti-Inositol Polyphosphate-5-Phosphatase, 72 KDa (INPP5E) (C-Term) antibody (ABIN2704925)

Western blot of 4T1 mouse breast carcinoma cell line extracts ($Inpp5e^{+/+}$, lane 1 and 2) and 4T1 Inpp5e CRISPR/Cas9 knock-out cell extracts ($Inpp5e^{em}$, lane 3 and 4) with ABIN2704925. A single band appears upon cell treatment with an inducer (unpublished) specifically in the $Inpp5e^{+/+}$ cells.