Datasheet for ABIN2777474

anti-GLI2 antibody (Middle Region)

2 Validations | 7 Images | 23 Publications

Go to Product page

Overview

Quantity:	100 µL
Target:	GLI2
Binding Specificity:	Middle Region
Reactivity:	Human
Host:	Rabbit
Clonality:	Polyclonal
Conjugate:	This GLI2 antibody is un-conjugated
Application:	Western Blotting (WB), Immunohistochemistry (IHC)

Product Details

Immunogen:	The immunogen is a synthetic peptide directed towards the N terminal region of human GLI2
Sequence:	RNDVHLRTPL LKENGDSEAG TEPGGPESTE ASSTSQAVED CLHVRAIKTE
Predicted Reactivity:	Human: 100%
Characteristics:	This is a rabbit polyclonal antibody against GLI2. It was validated on Western Blot and immunohistochemistry.
Purification:	Protein A purified

Target Details

Target:	GLI2
Alternative Name:	GLI2 (GLI2 Products)

Target Details

Background:	GLI2 encodes a protein which belongs to the C2H2-type zinc finger protein subclass of the Gli family. Members of this subclass are characterized as transcription factors which bind DNA through zinc finger motifs. These motifs contain conserved H-C links. Gli family zinc finger proteins are mediators of Sonic hedgehog (Shh) signaling and they are implicated as potent oncogenes in the embryonal carcinoma cell. The protein encoded by this gene localizes to the cytoplasm and activates patched Drosophila homolog (PTCH) gene expression. It is also thought to play a role during embryogenesis. The encoded protein is associated with several phenotypes- Greig cephalopolysyndactyly syndrome, Pallister-Hall syndrome, preaxial polydactyly type IV, postaxial polydactyly types A1 and B. Alias Symbols: HPE9, THP1, THP2 Protein Interaction Partner: SPOP, SKI, HDAC1, STK36, FBXW11, SUFU, ZIC2, ZIC1, CREB1, TBP, Protein Size: 1258
Molecular Weight:	133 kDa
Gene ID:	2736
NCBI Accession:	NM_030379, NP_084655
UniProt:	P10070
Pathways:	Hedgehog Signaling, Dopaminergic Neurogenesis

Application Details

Application Notes:	Optimal working dilutions should be determined experimentally by the investigator.
Comment:	Antigen size: 1258 AA
Restrictions:	For Research Use only

Handling

Format:	Liquid
Concentration:	Lot specific
Buffer:	Liquid. Purified antibody supplied in 1x PBS buffer with 0.09 % (w/v) sodium azide and 2 % sucrose.
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

Handling Advice:	Avoid repeated freeze-thaw cycles.
Storage:	-20 °C
Storage Comment:	For short term use, store at 2-8°C up to 1 week. For long term storage, store at -20°C in small aliquots to prevent freeze-thaw cycles.

Publications

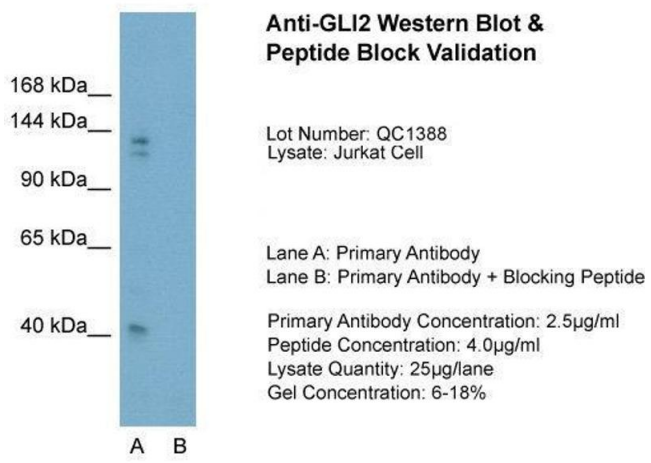
Product cited in:	Coyne, Gambling, Boucher, Carson, Johnson: "Role of claudin interactions in airway tight junctional permeability." in: **American journal of physiology. Lung cellular and molecular physiology**, Vol. 285, Issue 5, pp. L1166-78, (2003) (PubMed). There are more publications referencing this product on: Product page

Images

Western Blotting

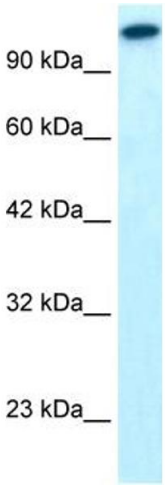
Image 1. In Fig. 6B the expression of human Gli2N is demonstrated. The GLI2 antibody (Middle Region, ABIN2777474) recognizes the human sequence RNDVHLRTPL LKENGDSEAG TEPGGPESTE ASSTSQAVED CLHVRAIKTE from human Gli2 in the middle region, which is also found in our Gli2N construct and therefore in the Gli2N expressing mouse models. In western blot analyses, this detection antibody can verify human Gli2N isoform as well as the full-length Gli2. In Figure 6B, all five mouse models show a band at the expected size for Gli2N, albeit the expression in hG::p53Fl/+ ;Gli2N+ and hG::NMYCF/l+;Gli2N+ mice was weaker compared to the corresponding homozygous mouse model and the hG::Gli2N+ mice (Fig. 6B). HEK293T cells were used as negative control and did not show any band as expected. MDA-MB-231 were used as positive control and showed, as expected, a band at the level of the full-length Gli2. Source: 10.1016/j.isci.2023.107501

Images



Western Blotting

Image 2. Host: Rabbit Target Name: GLI2 Sample Type: Jurkat Lane A: Primary Antibody Lane B: Primary Antibody + Blocking Peptide Primary Antibody Concentration: 2.5ug/mL Peptide Concentration: 4.0ug/mL Lysate Quantity: 25ug/lane Gel Concentration: 6%-18%



Western Blotting

Image 3. WB Suggested Anti-GLI2 Antibody Titration: 1.25ug/ml

ELISA Titer: 1:312500

Positive Control: Jurkat cell lysate

Please check the product details page for more images. Overall 7 images are available for ABIN2777474.

Validation report #101239 for Western Blotting (WB)

Successfully validated (Western Blotting (WB))

by Laboratory of Herditary Cancer, Division of Molecular Medicine, Rudjer Boskovic Institute

Report Number: 101239

Date: Oct 05 2017

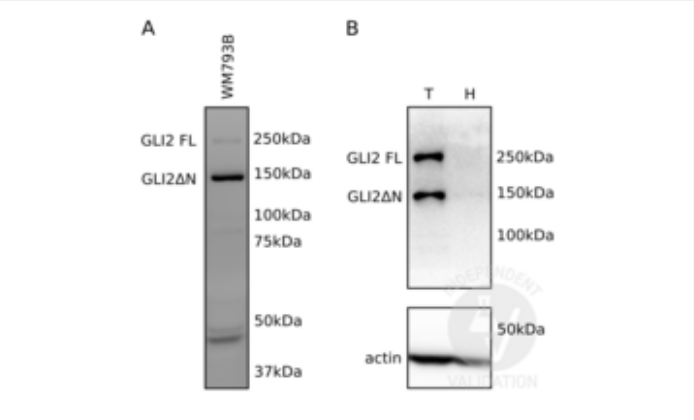
Target:	GLI2
Lot Number:	QC49260-42551
Method validated:	Western Blotting (WB)
Positive Control:	Human melanoma cell line WM793B Human metastatic melanoma tissue
Negative Control:	Human healthy skin not expressing GLI2
Notes:	Passed. ABIN2777474 specifically recognizes GLI2 in WM793B cells and human metastatic melanoma tissue.
Primary Antibody:	ABIN2777474
Secondary Antibody:	anti-rabbit IgG HRP-linked antibody (Cell Signaling Technology, 7074)
Protocol:	• Grow WM793B cells in RPMI (Lonza, BE12-702F) supplemented with FBS (Sigma-Aldrich, F7524) and penicillin/streptomycin (Thermo Fisher Scientific, 15070063), at 37°C and 5% CO_2 in a 10 cm TC-dish. • Wash cells 2x with PBS. • Harvest cells. • Lyse cells in 85µl cold lysis buffer (25mM Tris-HCl pH7.5, 150mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS) with sonication 2x 15s using an Labsonic M sonicator with a 1mm probe (B. Braun Biotech International, 8535027), 1 cycle, 80% amplitude. • Determine total protein content of the lysates using a BCA protein assay (Thermo Fisher Scientific, 23225). • Denature 50µg of total proteins for 5min at 95°C in Laemmli SDS sample buffer and subsequently separate them on a freshly cast denaturing 7% PAA gel (using components from Sigma-Aldrich) for 2h at 100V. • Transfer proteins onto Amersham Protran Premium 0.2 NC membrane (GE Healthcare, 10600004) with a Mini Trans-Blot cell (Bio-Rad, 170-3930) for 80min at 100V. • Block the membrane with blocking buffer and blocking reagent (TBST/5% non-fat milk) for 30min at RT. • Incubation with primary rabbit anti human GLI2 antibody (antibodies-online, ABIN2777474, lot QC492960-42551) diluted 1:1000 in TBST/5% non-fat milk ON at 4°C. • Wash membrane 3x for 5min with TBST.

- Incubation with secondary anti-rabbit IgG HRP-linked antibody (Cell Signaling Technology, 7074) diluted 1:3000 in 5% non-fat milk/TBST for 1h at RT.
- Wash membrane 3x for 5mins with TBST.
- Reveal protein bands using Super Signal West Pico and Super Signal West Femto reagents (Thermo Fisher Scientific) on an UVITEC Alliance Imaging system (Cambridge).

Experimental Notes:

- The GLI2 antibody ABIN2777474 reveals two protein bands of the expected molecular weight for the full-length (GLI2 FL) and the GLI2 N-terminally truncated (GLI2ΔN) isoform in lysates of WM793B cells.
- ABIN2777474 also detects both GLI2 FL as well as GLI2ΔN in metastatic melanoma cell lysates whereas neither protein is revealed in human healthy skin tissue in which expression of GLI2 is not to be expected.
- The predicted molecular weight of the full length GLI2 is 197kDa which is in accordance with our observation. With our previous anti-GLI2 antibody (sc-20291 (G-20), Santa Cruz Biotechnology) we also detected the GLI2ΔN protein at ~150kDa. The difference in apparent MW could be due to different migration of the marker.

Image for Validation report #101239



Validation image no. 1 for anti-GLI Family Zinc Finger 2 (GLI2) (Middle Region) antibody (ABIN2777474)

A Detection of endogenously expressed GLI2 with ABIN2777474 in total cell lysates of the human melanoma cell line WM793B. Both the full length protein (GLI2 FL) as well as the ΔN (GLI2ΔN) isoform are detected. B Detection of endogenously expressed GLI2 with ABIN2777474 in human metastatic melanoma tissue (T) compared to human healthy skin tissue that does not express GLI2 (H). Both the full length protein as well as the ΔN isoform are only detected in metastatic melanoma.

Validation report #102749 for Immunohistochemistry (IHC)

Successfully validated (Immunohistochemistry (IHC))

by Laboratory for Hereditary Cancer, Division of Molecular Medicine, Rudjer Boskovic Institute

Report Number: 102749

Date: Feb 02 2018

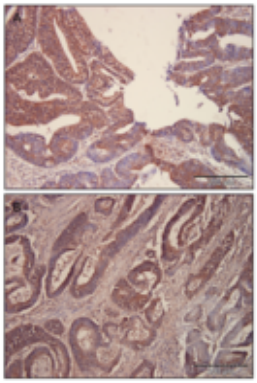
Target:	GLI2
Lot Number:	QC49260-42551
Method validated:	Immunohistochemistry (IHC)
Positive Control:	Human colon cancer tissue sections
Negative Control:	no primary antibody
Notes:	Passed. ABIN2777474 specifically labels the targeted antigen in human colon cancer samples in IHC.
Primary Antibody:	ABIN2777474
Secondary Antibody:	biotinylated anti-rabbit, streptavidin-HRP (LSAB2 System-HRP, Dako, K0675, lot 10126805)

Protocol:	• Fix human colon cancer tissue in 10% buffered formalin, ON at RT.
	• Process tissue using an automated tissue processor (Leica or Tissue-Tec VIP Sakura):
	  ○ 70% ethanol 2x for 30min.
	  ○ 96% ethanol 2x for 30min.
	  ○ Absolute ethanol 2x 30min.
	  ○ Xylene substitute (Tissue-Clear, Sakura) 2x 30min.
	  ○ Paraffin 2x 30min at 62°C. Embed tissue in paraffin blocks using the Tissue-Tec TEC embedding console system.
	• Cut paraffin embedded tissue with a Sliding microtome Slide 4003 E (PFM Medical) into 3µm sections.
	• Deparaffinize and rehydrate sections through a graded xylene and graded alcohol series:
	  ○ Bioclear New xylene substitute (Biognost, BCN-1L, lot BCN-03/17) 3x for 3min.
	  ○ 100% ethanol 2x for 1min.
	  ○ 95% ethanol 2x for 1min.
	  ○ 70% ethanol 2x for 1min.
	  ○ H_2O for 5min.
	• Incubate the sections in preheated citrate buffer (10mM Sodium Citrate, 0.05% Tween 20, pH6.0) preheated to 100°C for 20min.
	• Cool sections to RT for 20min.
	• Rinse sections 1x for 1min with 1x TBST.
	• Block endogenus peroxidase by incubating the sections in 3% H_2O_2 in methanol.

- Rinse sections 3x for 1min with 1x TBST.
- Block sections in Protein Block (Dako, X0909, lot 10070336) for 20min at RT.
- Blot excess serum from sections.
- Incubate sections with primary rabbit anti-human GLI2 antibody (antibodies-online, ABIN2777474, lot QC492960-42551) diluted 1:100 in 2% BSA/TBST ON at 4°C. Include a no primary antibody negative control.
- Wash sections 3x with TBST for 1min.
- Incubate sections with biotinylated anti-rabbit and anti-mouse immunoglobulin (LSAB2 System-HRP, Dako, K0675, lot 10126805) for 10min at RT.
- Wash sections 3x for 1min with TBST.
- Incubate sections with streptavidin-HRP (LSAB2 System-HRP, Dako, K0675, lot 10126805) for 10min at RT.
- Staining procedure with DAB chromogen (Dako, Liquid DAB+ Substrate Chromogen System, K3468, lot 10131855) and counterstaining with haematoxylin G2 (Biognost, HEMG2-OT-500, lot HEMG2-10/17).
- Rinse sections 1x for 5min with distilled water.
- Dehydrate in fresh ethanol:
  - 70% ethanol 2x for 1min.
  - 95% ethanol 2x for 1min.
  - 100% ethanol 2x for 1min.
  - Bioclear New (xylene substitute) 3x for 3min.
- Mount sections in Biomount New (Biognost, BMN-30, lot BMN-10/16) mounting medium.
- Dry sections in a ventilated place.
- Image acquisition onthe Olympus BX51 microscope at 200x magnification.

Experimental Notes:

- ABIN2777474 did not work with a protocol where the sections were incubated with epitope retrieval buffer at 85°C for 10min, and where the washing steps were done with PBS.
-
- Localization of GLI2 is cytoplasmic and nuclear. No signal was detected in the secondary antibody only control.



Validation image no. 1 for anti-GLI Family Zinc Finger 2 (GLI2) (Middle Region) antibody (ABIN2777474)

Immunohistochemical staining GLI2 with ABIN2777474 in well differentiated colorectal carcinoma (A) and poorly differentiated colorectal carcinoma (B). Localization of GLI2 is cytoplasmic and nuclear. Magnification 200x.