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Datasheet for 100-401-223

Gli1 Antibody

Overview

Description:	Anti-Gli1 (RABBIT) Antibody - 100-401-223
Item No.:	100-401-223
Size:	100 µL
Applications:	ELISA, IF, WB, ChIP, IHC, IP
Reactivity:	Human, Mouse
Host Species:	Rabbit

Product Details

Background:	Anti Gli1 Antibody was produced against a peptide corresponding to the carboxy-terminal region of the mouse Gli-1 protein. This region of Gli1 is not conserved among other gli family members, namely Gli-2 and Gli-3. Gli was termed by Kinzler et al. (1987) as 'glioma-associated oncogene' amplified in malignant gliomas. Analysis of the cloned gene demonstrates that the gene contains 5 repeats of zinc-finger sequences, which places Gli in the family of Kruppel (Kr) zinc finger proteins. Northern analysis reveals that Gli is expressed in embryonal carcinoma cells but not in most adult tissue. Gli has been localized to 12q13-q14.3 by Southern blot analysis. In mice, the gene is located on chromosome 10. In mice, three zinc finger transcription factors, Gli-1, Gli-2 and Gli-3, have been implicated in the transduction of Sonic hedgehog (Shh) signal. In papillary epithelium, shh, gli1 and ptc all follow similar expression patterns. Gli-1 expression is central and probably sufficient for tumor development in humans.
Synonyms:	rabbit anti-Gli-1 Antibody, rabbit anti-Gli1 Antibody, Zinc finger protein GLI1 antibody, Glioma-associated oncogene antibody, Oncogene GLI antibody
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	Antiserum

Target Details

Gene Name:	Gli1
Reactivity:	Human, Mouse

Immunogen Type:	Conjugated Peptide
Immunogen:	The whole rabbit serum was prepared by repeated immunizations with a synthetic peptide corresponding to amino acids 805-820 of mouse Gli-1. The peptide was synthesized as a multiple antigen peptide (MAP).
Purity/Specificity:	This whole rabbit antiserum was prepared by delipidation and defibrination followed by the addition of buffer salts and preservative. Reactivity is observed against Mouse and Human Gli-1. Cross-reactivity with other species is likely but has not been determined. No reaction occurs with human or mouse Gli-2 or Gli-3.
Relevant Links:	• UniProtKB - P47806 • NCBI - 4885279 • GeneID - 2735 • GeneID - 14632

Application Details

Tested Applications:	ELISA, IF, WB
Suggested Applications:	ChIP, IHC, IP (Based on references)
Application Note:	This antibody has been tested for use in ELISA, immunofluorescence, and western blot. Specific conditions for reactivity should be optimized by the end user. Expect a band approximately 120 kDa in size corresponding to Gli-1 protein by western blotting in the appropriate cell lysate or extract. For immunohistochemistry, perform heat mediated antigen retrieval via the microwave method before commencing with staining.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:20,000 - 1:100,000
IF:	1:500 - 1:2,000
IHC:	1:500 - 1:2,000
WB:	1:2,000 - 1:10,000

Formulation

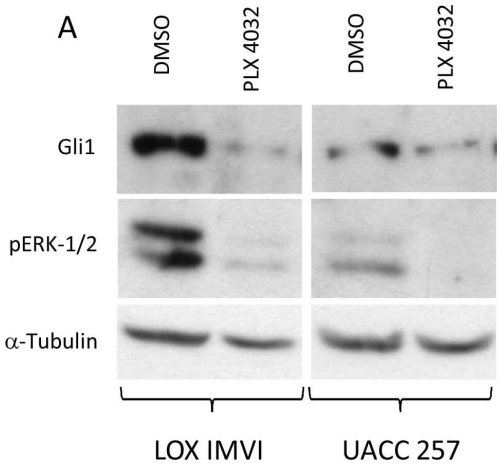
Physical State:	Liquid (sterile filtered)
Concentration:	75 mg/mL by Refractometry
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide

Stabilizer:	None

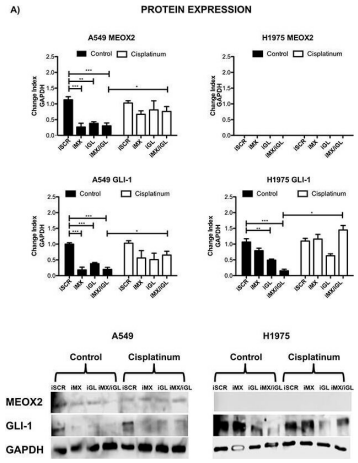
Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
Expiration:	Expiration date is one (1) year from date of receipt.

Images

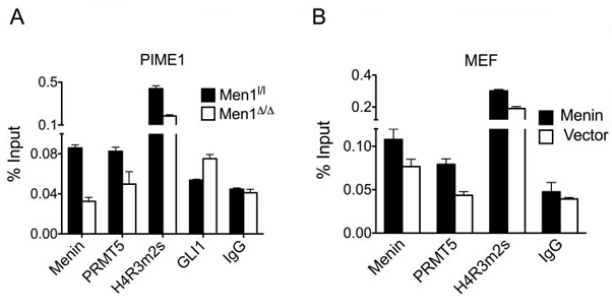


Western Blot
Inhibition of BRAFV600E, expression of GLI1 and SHH-GLI pathway inhibition by NVP-LDE225 in human melanoma cells in vitro. A) LOX IMVI and UACC 257 with PLX-4032 at the dose of 1 µM for 24 hr. subsequently protein lysates prepared and subjected to WB analysis for the expression of GLI1 and phospho-ERK1/2. B) Effect of NVP-LDE225 on PTCH1 promoter. In total, 1 µg of PTCH1 pGL3b-hPTCH1-prom-wt or pGL3b-hPTCH1-prom-mut luciferase construct and reporter were cotransfected into LOX IMVI cells. Cells were subsequently treated with 10 µM of NVP-LDE225 or cyclopamine for 4 hours (time point selected base on kinetic experiments). Fold activation was calculated relative to cells transfected with 3 µg of pB-actin-RL. One representative experiment of 2 is shown. Figure provided by CiteAb. Source: PLoS One, PMID: 23935925.



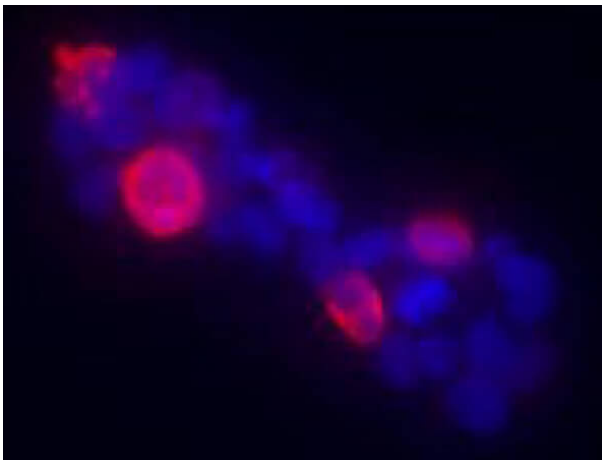
Western Blot

Inducible MEOX2-GLI1 axis expression was involved in cellular migration and cellular proliferation in lung cancer cells(A) A549 and H1975 lung cancer cells demonstrated an inducible GLI-1 protein expression pattern following treatment with 8 µM cisplatinum and reduced GLI-1 inducible expression following the application of specific anti-MEOX2 siRNA and/or anti-MEOX2 siRNA plus anti-GLI1 siRNA in the presence of 8 µM cisplatinum. Western blot statistical analyses, assessed via quantitative densitometry, were performed to determine *p≤0.05 by one-way ANOVA and Dunnett's test for multiple comparisons to identify significant differences with respect to controls. Student's t-test was performed to identify significant differences between control and cisplatinum treatment. Quantification analyses were normalized to scrambled siRNA as a negative control for gene silencing. Images were obtained using a C-DIGIT device (LICOR), and pixel quantification and data analyses were carried out using Image Studio software. Total pixel intensity for each specific protein product was normalized to GAPDH. (B) Cell culture images and graphs showing the quantitative analysis of cellular migration as a percentage (transwell migration assays) indicated significant MEOX2 and GLI-1 protein-dependent functions following the individual and combined application of anti-MEOX2 and anti-GLI1 siRNAs in A549, NH2347 and H1975 lung adenocarcinoma cells; **p≤0.005 and ***p≤0.0001 based on one-way ANOVA and Dunnett's multiple comparisons test. (C) Cell culture images and graphs showing the quantitative analysis of cellular proliferation (clonogenic assays) indicated significant MEOX2 and GLI-1 protein-dependent functions following the individual and combined application of anti-MEOX2 and anti-GLI1 siRNAs in A549, NH2347 and H1975 lung adenocarcinoma cells; **p≤0.005 and ***p≤0.0001 based on one-way ANOVA and Dunnett's multiple comparisons test. Transwell migration index and colony growth (clonogenic assays) rates were normalized and quantified using the ImageJ Colony Number plugin tool (see Materials and Methods). Figure provided by CiteAb. Source: Oncotarget, PMID: 28978016.



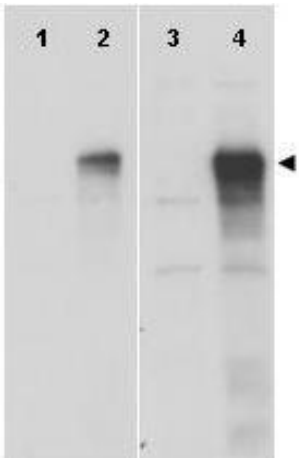
ChIP

Menin regulates Gli1 by recruiting PRMT5 and increasing symmetric histone H4 arginine dimethylation (H4R3m2s) at the Gli1 promoter.
(A) Chromatin immunoprecipitation (ChIP) using antibodies against menin, PRMT5, H4R3m2s, GLI1 and control IgG at the Gli1 promoter in either DMSO (Men1l/l) or tamoxifen-treated Men1l/l; CreER (Men1Δ/Δ) PIME1 cells.
(B) ChIP with antibodies against menin, PRMT5 and H4R3m2s at the Gli1 promoter in Men1-null MEFs complemented with either vector or wild type menin. Error bars indicate ± s.d.
Figure 3. PMID: 23928057.



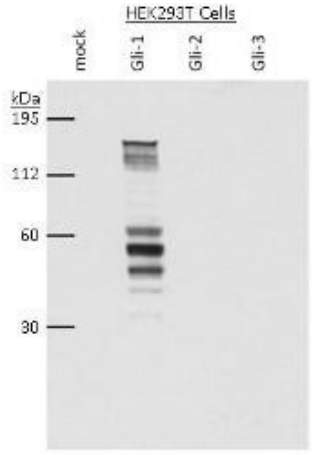
Immunofluorescence Microscopy

Immunofluorescence of Anti-Gli-1 antibody. HEK293T cells were transiently transfected with Gli-1 (murine). Primary Antibody: Rockland's Anti-Gli-1 antiserum (rabbit) was added 1:400. Secondary Antibody: fluorescent labeled anti-rabbit IgG. Personal communication, Tom Curran, Children's Hospital of Philadelphia, Philadelphia, PA. Detection of mouse Gli-1 present in transfected 293T cells (red).



Western Blot

Western blot using Rockland's anti-Gli-1 antibody shows detection of a band at ~150 kDa (arrowhead) corresponding to human Gli-1 present in transfected 293T cell lysates (lanes 2 and 4). Mock 293T cell lysates with vector only show no staining (lanes 1 and 3). Lysates were separated by SDS-PAGE and transferred to nitrocellulose. After blocking the membrane was probed with the primary antibody diluted to 1:8,000 (lanes 1 and 2) or 1:4,000 (lanes 3 and 4). Molecular weight estimation was made by comparison to MW markers. Personal communication, Hiro Kimura, St. Jude Children's Research Hospital, Memphis, TN.



Western Blot
Western blot using Rockland's anti-Gli-1 antibody shows detection of a band at ~150 kDa corresponding to Gli-1 present in HEK293T whole cell lysate transiently transfected with Gli-1 (lane 2). Mock 293T cell lysates and 293T cell lysates transfected with Gli-2 and Gli-3 show no staining (lanes 1, 3 and 4 respectively). Lysates were separated by SDS-PAGE and transferred to nitrocellulose. After blocking, the membrane was probed with Anti-Gli-1 antiserum diluted 1:5,000, followed by HRP conjugated donkey anti-rabbit (Rockland p/n 611-7302). Personal communication, Tom Curran, Children's Hospital of Philadelphia, Philadelphia, PA.

References

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