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Datasheet for 600-401-694

GLI3 Antibody

Overview

Description:	Anti-GLI3 (RABBIT) Antibody - 600-401-694
Item No.:	600-401-694
Size:	100 µg
Applications:	ELISA, IF, IHC, Multiplex, WB
Reactivity:	Human
Host Species:	Rabbit

Product Details

Background:	Gli-3 (also known as Zinc Finger Protein Gli-3 or GLI-Kruppel family member GLI-3) belongs to the GLI C2H2-type zinc-finger protein family and contains 5 C2H2-type zinc fingers. Gli-3 is very important for normal limb and brain development and is implicated in the transduction of Shh signal. Gli-3 is a nuclear protein expressed in a wide variety of normal adult tissues, including lung, colon, spleen, placenta, testis, and myometrium. Defects in Gli-3 are the cause of Greig cephalo-poly-syndactyly syndrome (GCPS); an autosomal dominant disorder-affecting limb and cranio-facial development. Two isoforms of human Gli-3 have been reported. One is the full-length protein at ~170-190kDa and the other is a truncated isoform at ~80kDa.
Synonyms:	Rabbit anti-GLI-3 antibody, Transcriptional activator GLI3, Gli 3, GLI3 form of 190 kDa, GLI3 form of 83 kDa
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	GLI3
Reactivity:	Human
Immunogen Type:	Conjugated Peptide

Immunogen:	This affinity purified antibody was produced from monospecific rabbit serum by repeated immunizations with a synthetic peptide corresponding to an internal region near amino acids 30 -60 of human Gli-3 protein.
Purity/Specificity:	This affinity-purified antibody is directed against human Gli-3 protein. The product was affinity purified from monospecific antiserum by immunoaffinity purification. A BLAST analysis was used to suggest cross reactivity with Gli-3 from human, chimpanzee, squirrel monkey, Xenopus laevis, chicken, dog and quail based on 100% sequence homology with the immunogen. Reactivity is also expected against homologues from mouse (94%) and rat (88%) based on partial homology. Reactivity with Gli-3 from other sources is not known.
Relevant Links:	• NCBI - 119393899 • UniProtKB - P10071 • GeneID - 2737

Application Details

Tested Applications:	ELISA, IF, IHC, Multiplex, WB
Application Note:	This antibody has been tested for use in ELISA, immunohistochemistry, immunofluorescence, and western blot. Specific conditions for reactivity should be optimized by the end user. Detection of Gli-3 by western blot may be enhanced if nuclear extracts are used instead of whole cell lysates as the expression/abundance of Gli-3 is likely to be low. Furthermore, Gli3 expression is likely to be developmentally regulated and induced, making it difficult to detect in whole tissue homogenates.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:6,000 - 1:30,000
IF:	User Optimized
IHC:	0.5 mg/ml - 5 µg/ml
WB:	1:500 - 1:2,000

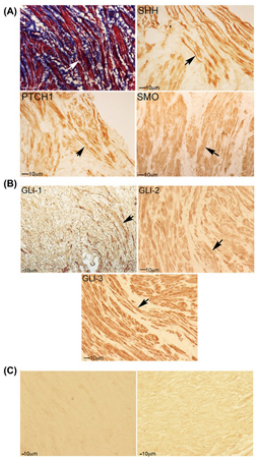
Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	1.14 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None

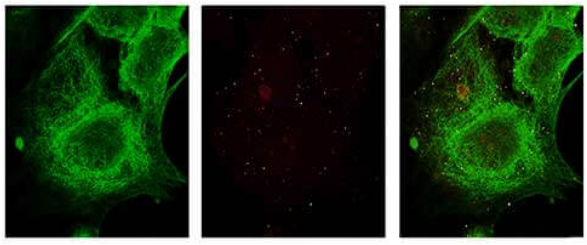
Shipping & Handling

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.

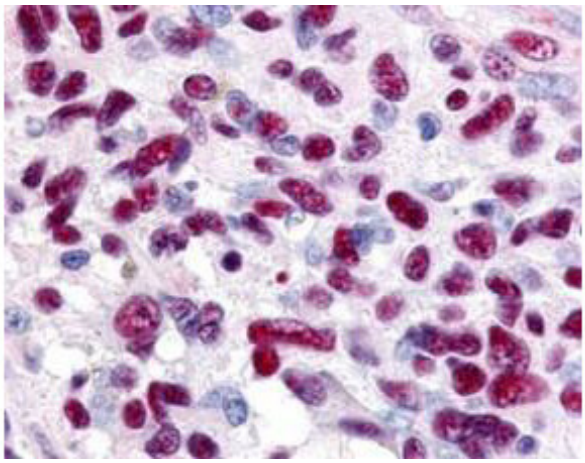
Images



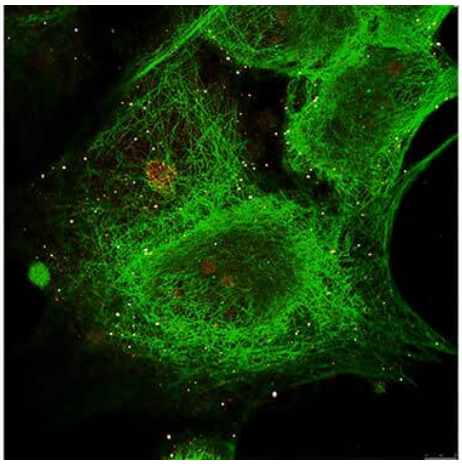
Immunohistochemistry
Trichrome stain and IHC analysis for SHH pathway in human RS muscle. A, Trichrome stain of human RS indicates abundant muscle and collagen. Arrows indicate muscle. IHC analysis of human RS shows SHH, PTCH1, and SMO protein localization in RS muscle. B, GLI-1, GLI-2 (p/n 600-401-695), and GLI-3 (p/n 600-401-694) are abundant in RS muscle. C, No primary controls (left: mouse anti-rabbit, and right: donkey anti-goat) are presented. Arrows indicate protein. 100–200× magnification. Fig 1. PMID: 30187971



Immunofluorescence Microscopy
Immunofluorescence Microscopy of Rabbit anti-Gli-3 antibody. Tissue: MCF-7 cell. Antigen retrieval: not required. Primary antibody: Gli-3 antibody and Anti-alpha-Tubulin at 5 µg/mL for 1 h at RT. Secondary antibody: Fluorescein secondary antibody at 1:10,000 for 45 min at RT. Localization: Gli-3 is nuclear. Staining: Image (1) shows alpha-Tubulin staining as green fluorescent signal. Image (2) shows Gli-3 staining as red fluorescent signal and Images (3) shows both antibodies fluorescing using STED microscopy.



Immunohistochemistry
Immunohistochemistry of Rabbit anti-Gli-3 antibody. This image tissue: human glioblastoma. Specific staining was also noted in tissue from adrenal, brain, glioblastoma, colon, heart, kidney, lung, liver, skeletal muscle, ovary, pancreas, placenta, skin, spleen, stomach, testes, thymus, thyroid, tonsil and uterus. Fixation: formalin fixed paraffin embedded. Antigen retrieval: not required. Primary antibody: Gli-3 antibody at 0.625 µg/ml for 1 h at RT. Secondary antibody: Peroxidase rabbit secondary antibody at 1:10,000 for 45 min at RT. Localization: Gli-3 is nuclear and smooth muscle. Staining: Gli-3 as precipitated red signal with hematoxylin purple nuclear counterstain.



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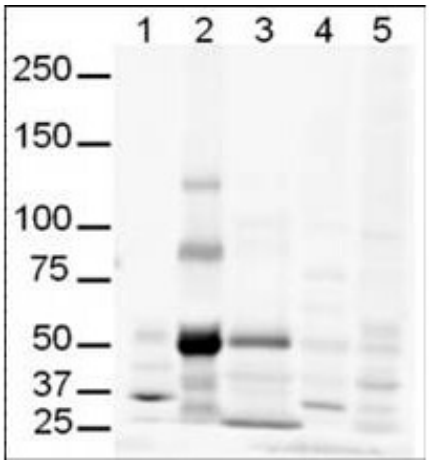
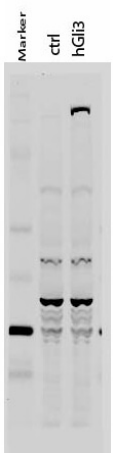


Western Blot
Western Blot of Rabbit anti-Gli-3 antibody. Lane 1: human brain whole cell lysate. Lane 2: human lung whole cell lysate. Lane 3: human spleen whole cell lysate. Lane 4: mouse brain whole cell lysate (p/n W10-000-T004). Lane 5: mouse lung whole cell lysate (p/n W10-000-MQ1). Load: 20 µg per lane. Primary antibody: Gli-3 antibody at 1:500 for overnight at 4°C. Secondary antibody: IRDye800™ rabbit secondary antibody at 1:10,000 for 45 min at RT. Block: 5% BLOTTO overnight at 4°C. Predicted/Observed size: Isoforms at ~170-190kDa and ~80kDa. Lane 2 shows what may be truncated Gli-3 (~80kDa). Other band(s): The strong band at ~50 kDa is unknown.



Western Blot
Western Blot of Rabbit anti-Gli-3 antibody. Lane 1: 50 kDa molecular weight marker. Lane 2: 293T cells transfected with CrkL-Flag. Lane 3: 293T cells transfected with human Gli-3. Load: 35 µg per lane. Primary antibody: Gli-3 antibody at 1:400 for overnight at 4°C. Secondary antibody: IRDye800™ rabbit secondary antibody at 1:10,000 for 45 min at RT. Block: 5% BLOTTO overnight at 4°C.
Predicted/Observed size: 170-190 kDa for hGli-3. Other band(s): Non specific background ~60kDa.

References

- Hehemann M et al. Sonic hedgehog regulation of human rhabdosphincter muscle:Potential implications for treatment of stress urinary incontinence. *Neurourol Urodyn.* (2018)

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