

Certificate of Analysis

Product: Affinity Purified Anti-Human Gli-3 [Rabbit]

Code: 600-401-694

Lot #: 20161

Size: 100 µg

Physical State: Liquid (sterile filtered)

Antibody Concentration: 0.84 mg/ml (by UV absorbance at 280 nm)

Buffer: 0.02 M Potassium Phosphate, 0.15 M NaCl, pH 7.2

Stabilizer: None

Preservative: 0.1% (w/v) Sodium Azide

Storage Conditions: Store vial at -20° C prior to opening. For extended storage aliquot contents and freeze at -20° C or below. Avoid cycles of freezing and thawing. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use. Expiration date is one (1) year from date of opening.

Background Information: 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.

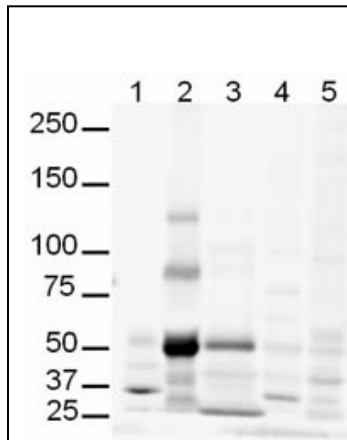


Figure. Western blot using Rockland's anti-Gli-3 antibody shows detection of multiple bands in human lung lysate believed to be Gli-3. Lanes contain 20 µg of whole cell lysates from 1 - human brain, 2 - human lung, 3 - human spleen, 4 - mouse brain and 5 - mouse lung. While no recognizable staining can be seen on mouse tissue, human lung shows what may be truncated Gli-3 (~80kDa). This identity of the strong band at ~50 kDa is unknown. After blocking, the membrane was probed with the primary antibody diluted to 1:500. For detection use HRP Gt-a-Rabbit IgG (611-103-122). 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.

Recommended Dilutions:

ELISA	1:6,000 - 1:30,000
WESTERN BLOT	1:500 - 1:2,000
IMMUNOHISTOCHEMISTRY	0.5 µg/ml - 5 µg/ml
OTHER APPLICATIONS	User Optimized

Application Note(s): This antibody has been tested for use in ELISA, immunohistochemistry and western blot. Specific conditions for reactivity should be optimized by the end user. See figure legend for expectations by western blot.

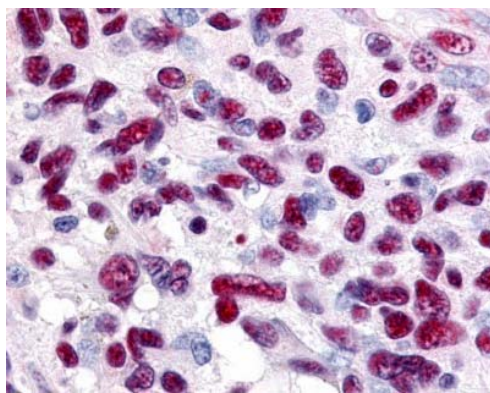


Figure 2. Immunohistochemistry. Rockland's Affinity Purified anti-Human Gli-3 antibody was used at a 0.625 µg/ml to detect Gli-3 in a variety of tissues. Strong nuclear and smooth muscle staining was noted to be consistent with previously published reports. Specific staining was noted in tissue from adrenal, brain, glioblastoma, colon, heart, kidney, lung, liver, skeletal muscle, ovary, pancreas, placenta, skin, spleen, stomach, testes, thymus, thyroid, tonsil and uterus. This image shows Gli-3 staining of human glioblastoma. Tissue was formalin-fixed and paraffin embedded.
Personal Communication, Tina Roush, LifeSpanBiosciences, Seattle, WA.

Purity and 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 Link(s): Swiss Prot: [P10071](#)

NCBI Link [NP_000159](#)

Immunogen: This affinity purified antibody was produced from monospecific rabbit serum by repeated immunizations with a synthetic peptide corresponding to amino acids 41-57 of human Gli-3 protein.

Protein Sequence: Human Gli-3, 1580 aa, predicted MW 170.0 kDa

1	meaqshsst	tekkkvensi	vkcstrdvs	ekavasstts	nedespgqty	hrerrnaitm
61	qqnqvqglsk	vseepstssd	eraslikkei	hgslphvaep	svpyrgtvfa	mdprngymep
121	hyhpphlfpa	fhppvpidar	hhegyryhdp	spipplhmts	alsssptypd	lpfirisphr
181	npaaasespf	spphpyinpy	mdyirslhss	pslsmisatr	glsptdapha	gvspaeyyhq
241	malltgqrsp	yadiipsaat	agtgaihmev	lhamdstfrs	sprlsarpsr	krtlsispls
301	dhsfdlqtm	rtspnslvti	Innsrssssa	sgsyghlsas	aispalsfty	ssapvslhmf
361	qqilsrqsl	gsafghsppl	ihpaptftq	rpigiptvl	npvqvssgps	essqnkptse
421	savsstgdp	hnrskikpd	edlpstgarg	qqeqpegttl	vkeegdkdes	kqepviyet
481	nchwegcare	fdtqeqlvhh	innndihgek	kefvcrlwdc	sreqkpfkaq	ymlvvhmrrh
541	tgekphkctf	egctkaysrl	enlkthlrsh	tgekpyvceh	egcnkafnsa	sdrakhqnr
601	hsnekpyvck	ipgctkrytd	psslrkhvkt	vhgpeahvkt	kqrgdihprp	ppprdsghs
661	qsrspgrptq	galgeqqdls	ntskreelc	qvktvkaekp	mtsqpspggq	sscssqqspi
721	snysnsgl	pltdggsigd	lsaidetpim	dstistatta	lalqarrnpa	gtkwmehrvk
781	erlkqvnqmf	prlnpilppk	apavsplign	gtqsnntcsl	ggpmtllpgr	sdlsgvdtm
841	lnmlnrrdss	astissayls	srrssgispc	fsrrsseas	qaegrpqnvs	vadsydpist
901	dasrrsseas	qsdglpslls	ltpaqyrlk	akyaatggp	pptlpnmer	mslklrall
961	gdalepgrval	ppvhaprrcs	dggahgygr	hlqphdalgh	gvrrasdpr	tgsegalpr
1021	vprfsslsc	nppamatsae	krslvlqnyt	rpeggqsmf	hsspcpsit	envtiesltm
1081	dadanlnded	flpddvvqyl	nsqnqagyey	hfpsalpdds	kvphgpgdfd	apglpdsghg
1141	qqfhaleqpc	pegsktldpi	qwnvssgsa	dlsssklkcq	prpavpqttra	fgfcngmvvh
1201	pqnplrsqpa	pqnplrsqpa	ngygppehlm	lhnspsgsts	gnafheqpc	apqygnclnr
1261	qpvpagaldg	acgagiqask	lkstpmqsg	gqlnfglpva	pnesagsmvn	gmqnqdpvgq
1321	gylahqilgd	smqhpqagrp	gqqmlgqisa	tshiniyqgp	esclpgahgm	gspsslavv
1381	rgyqpcasfg	gsrrqamprd	slalqsgqls	dstqtrcvng	ikmemkgqph	plcslnlqns
1441	qqfydqtvgf	sqdtkagsf	sisdascllq	gtsaknsell	spganqvst	vdsldshdle
1501	gvqidfdaii	ddgdhsslms	galspsiiqn	ishssrrltt	praslpfpal	smstnmaig
1561	dmssltsla	eeskflavmq				

Background Reference(s):

Johnston, J.J., et al. (2005) Molecular and clinical analyses of Greig cephalopolysyndactyly and Pallister-Hall syndromes: robust phenotype prediction from the type and position of GLI3 mutations. *Am. J. Hum. Genet.* **76** (4), 609-622.

Mendoza-Londono, R., Kashork, C.D., Shaffer, L.G., Krance, R. and Plon, S.E. (2005) Acute lymphoblastic leukemia in a patient with Greig cephalopolysyndactyly and interstitial deletion of chromosome 7 del(7)(p11.2 p14) involving the GLI3 and ZNFN1A1 genes. *Genes Chromosomes Cancer* **42** (1), 82-86.

Johnston, J.J., Olivos-Glander, I., Turner, J., Aleck, K., Bird, L.M., Mehta, L., Schimke, R.N., Heilstedt, H., Spence, J.E., Blancato, J. and Biesecker, L.G. (2003) Clinical and molecular delineation of the Greig cephalopolysyndactyly contiguous gene deletion syndrome and its distinction from acrocallosal syndrome. *Am. J. Med. Genet.* **123** (3), 236-242.

Related Products:

[#100-401-223](#) Anti-Gli-1 (Rabbit)
[#600-401-845](#) Affinity Purified Anti-Human Gli-2 (Rabbit)
[#600-401-695](#) Affinity Purified Anti-Mouse Gli-2 (Rabbit)
[#600-401-694](#) Affinity Purified Anti-Human Gli-3 (Rabbit)
[#611-703-127](#) Peroxidase Conjugated Affinity Purified Anti-RABBIT IgG (H&L) (DONKEY) MX10
[#611-132-122](#) IRDye800 Conjugated Affinity Purified Anti-RABBIT IgG (H&L) (GOAT) MX10
[#MB-070](#) Blocking Buffer for Fluorescent Western Blotting
[#KIA-003](#) **MaxTag™** Anti-RABBIT IgG Kit for Immunoblotting

Note: This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information.

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