

DESCRIPTION

Species Reactivity	Human/Mouse
Specificity	Detects human and mouse GLI-3 in direct ELISAs and Western blots. In direct ELISAs and Western blots, less than 1% cross-reactivity with recombinant human (rh) GLI-1 and rhGLI-2 is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	<i>E. coli</i> -derived recombinant human GLI-3 Met1-Glu479 Accession # P10071
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied as a 0.2 µm filtered solution in PBS.

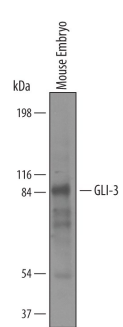
APPLICATIONS

Please Note: Optimal dilutions should be determined by each laboratory for each application. *General Protocols* are available in the *Technical Information* section on our website.

	Recommended Concentration	Sample
Western Blot	1 µg/mL	See Below
Chromatin Immunoprecipitation (ChIP)	5 µg/10 ⁶ cells	See Below
Immunocytochemistry	5-15 µg/mL	See Below

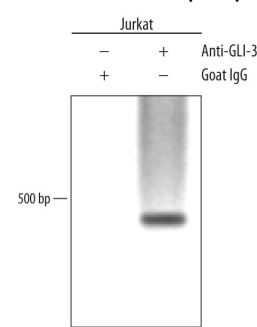
DATA

Western Blot



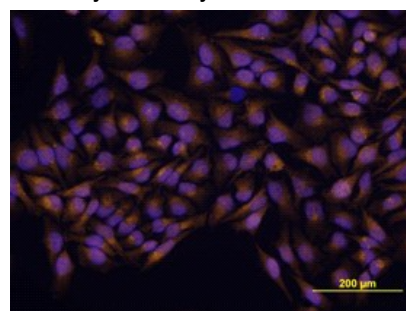
Detection of Mouse GLI-3 by Western Blot. Western blot shows lysates of mouse embryo tissue. PVDF membrane was probed with 1 µg/mL of Goat Anti-Human/Mouse GLI-3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3690) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF019). A specific band was detected for GLI-3 at approximately 85 kDa (as indicated). This experiment was conducted under reducing conditions and using *Immunoblot Buffer Group 8*.

Chromatin Immunoprecipitation (ChIP)



Detection of GLI-3-regulated Genes by Chromatin Immunoprecipitation. Jurkat human acute T cell leukemia cell line treated with 50 ng/mL PMA and 200 ng/mL calcium ionomycin for 30 minutes was fixed using formaldehyde, resuspended in lysis buffer, and sonicated to shear chromatin. GLI-3/DNA complexes were immunoprecipitated using 5 µg Goat Anti-Human/Mouse GLI-3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3690) or control antibody (Catalog # AB-108-C) for 15 minutes in an ultrasonic bath, followed by Biotinylated Anti-Goat IgG Secondary Antibody (Catalog # BAF109). Immunocomplexes were captured using 50 µL of MagCelect Streptavidin Ferrofluid (Catalog # MAG999) and DNA was purified using chelating resin solution. The *gli-1* promoter was detected by standard PCR.

Immunocytochemistry



GLI-3 in HeLa Human Cell Line. GLI-3 was detected in immersion fixed HeLa human cervical epithelial carcinoma cell line using Human/Mouse GLI-3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3690) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Goat IgG Secondary Antibody (yellow; Catalog # NL001) and counterstained with DAPI (blue). View our protocol for *Fluorescent ICC Staining of Cells on Coverslips*.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	Use a manual defrost freezer and avoid repeated freeze-thaw cycles. <ul style="list-style-type: none"> ● 12 months from date of receipt, -20 to -70 °C as supplied. ● 1 month, 2 to 8 °C under sterile conditions after reconstitution. ● 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

Human GLI-3 is a 190 kDa transcription factor of the C2H2-type zinc-finger protein family. GLI-3 appears to play a role in limb and brain development, probably through sonic hedgehog signaling. GLI-3 is regulated by PKA-mediated phosphorylation. Unphosphorylated full-length GLI-3 is a transcriptional activator, while phosphorylated GLI-3 is cleaved after amino acid 700 to generate an N-terminal 83 kDa transcriptional repressor. Over the region used as immunogen, human GLI-3 is 95% identical to the corresponding mouse and canine protein sequences.