Human/Mouse/Hamster GDF-9 Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF739

DESCRIPTION

Species Reactivity	Human/Mouse/Hamster
Specificity	Detects mouse GDF-9 in direct ELISAs and human, mouse and hamster GDF-9 in Western blots. In direct ELISAs, less than 1% cross-reactivity with recombinant mouse (rm) GDF-1, rmGDF-5, rmGDF-6, rmGDF-8, and rmGDF-15 is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	*E. coli*-derived recombinant mouse GDF-9
Formulation	Lyophilized from a 0.2 μm filtered solution in PBS with Trehalose.

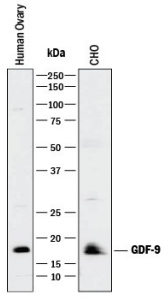
APPLICATIONS

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
Immunohistochemistry	5-15 µg/mL	See Below

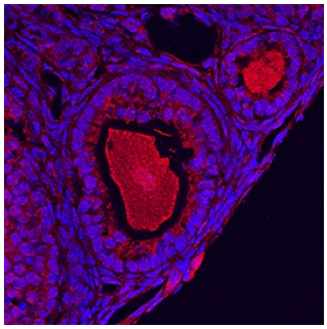
DATA

Western Blot



Detection of Human/Hamster GDF-9 by Western Blot. Western blot shows lysates of human ovary tissue and CHO Chinese hamster ovary cell line. PVDF membrane was probed with 1 µg/mL of Goat Anti-Human/Mouse/Hamster GDF-9 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF739) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). A specific band was detected for GDF-9 at approximately 18 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunohistochemistry



GDF-9 in Mouse Ovary. GDF-9 was detected in perfusion fixed frozen sections of mouse ovary using Goat Anti-Human/Mouse/Hamster GDF-9 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF739) at 5 µg/mL overnight at 4 °C. Tissue was stained using the NorthernLights™ 557-conjugated Anti-Goat IgG Secondary Antibody (red; Catalog # NL001) and counterstained with DAPI (blue). Specific staining was localized to developing oocytes. View our protocol for Fluorescent IHC Staining of Frozen Tissue Sections.

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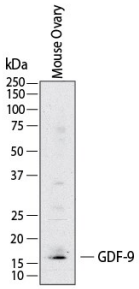
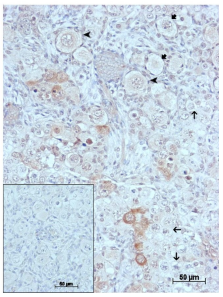
Western Blot



Detection of Mouse GDF-9 by Western Blot. Western Blot shows lysates of mouse ovary. PVDF membrane was probed with 1 µg/ml of Goat Anti-Human/Mouse/Hamster GDF-9 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF739) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). A specific band was detected for GDF-9 at approximately 17 kDa (as indicated). This experiment was conducted under reducing conditions and using Western Blot Buffer Group 1.

Immunohistochemistry-Paraffin

F



Detection of Human GDF-9 by Immunohistochemistry-Paraffin GDF9 is expressed in the human fetal ovary.qRT-PCR analysis of GDF9 (A), BMP15 (B) and NOBOX (C) mRNA expression in human fetal ovary across the gestational range of 8 to 20 weeks. Ovaries (n = 5–7 per group) were grouped according to developmental stage and transcript levels measured relative to those of RPL32. Bars indicate mean±sem. Statistically different levels are indicated by asterisks above the columns, thus expression of GDF9 at 18–20 weeks was significantly higher than at 8–11 weeks ($p<0.005$) as was expression of BMP15 and of NOBOX (both $p<0.01$). DAB immunohistochemical detection of GDF9: 19 week (D, E) and 20 week (F) human fetal ovary stained with anti-GDF9 antibody or normal goat IgG negative control (F inset)—positive staining is brown. Thick arrows indicate primordial follicles and thin arrows germ cells that are not stained for GDF9 while the arrowheads indicate primordial follicles that are positive for GDF9. Scale bars are 50µm (D and F) and 20µm (E). Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/25790371), licensed under a CC-BY license. Not internally tested by R&D Systems.

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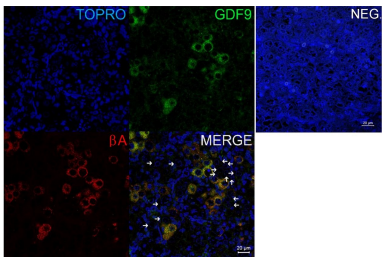
Immunocytochemistry/ Immunofluorescence

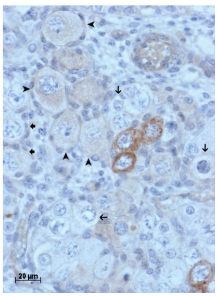
A



Detection of Human GDF-9 by Immunocytochemistry/ Immunofluorescence Co-localisation of GDF9 with activin βA but not DAZL or BOLL prior to follicle formation.(A) Double immunohistochemistry of 18 week fetal ovary stained for GDF9 (green) and activin βA (red), thus in the merged image co-expression is yellow. Unstained germ cells are indicated with arrows. Counterstain is TOPRO. (B) Triple fluorescent immunohistochemistry for GDF9 (green), DAZL (blue) and BOLL (red) in 20 week human fetal ovary with DAPI as counterstain (grey). Split channel and merged images in (A) and (B) are shown as are merged images of non-immune serum negative control (NEG). Scale bars are 20µm. (C) Nuclear diameters of DAZL, BOLL and GDF9 stained germ cells indicates that GDF9 positive cells are significantly larger ($p<0.001$) than DAZL but not BOLL expressing cells (bars indicate mean ± sem). (D) Higher magnification merged image of GDF9/DAZL/BOLL immunohistochemistry showing one large primordial follicle is positive for both GDF9 and DAZL but other follicles are positive only for DAZL. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/25790371), licensed under a CC-BY license. Not internally tested by R&D Systems.

Immunohistochemistry-Paraffin

E



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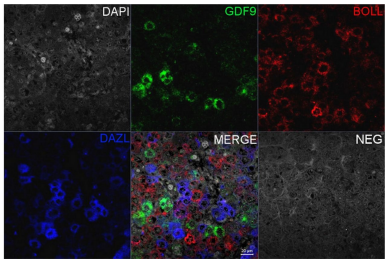

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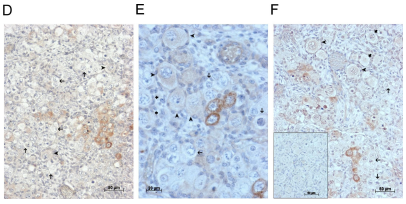
Immunohistochemistry



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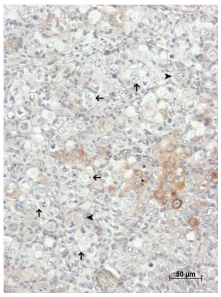
Immunohistochemistry-Paraffin

D



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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.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 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.

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BACKGROUND

Growth differentiation factor-9 (GDF-9) is a member of the transforming growth factor-β (TGF-β) superfamily, and is an oocyte secreted paracrine factor essential for mammalian ovarian folliculogenesis (1-2). Mouse GDF-9 is synthesized as a 441 amino acid (aa) prepropeptide that contains a 29 aa signal sequence, a 277 aa propeptide, and a 135 aa mature chain. Residues 340-441 constitute a TGF-β like domain. In addition, there is one potential site of N-linked glycosylation in the mature chain. Unlike other members of the TGF-β superfamily, GDF-9 lacks the conserved cysteine residue that is believed to form the sole disulfide linkage between subunits in other family members (3). Mature mouse GDF-9 shares 90% aa sequence identity with mature human GDF 9. The protein is expressed throughout the development of the maturing follicle (2). GDF-9 functions as a paracrine factor in the regulation of granulosa cell proliferation and differentiation, and is essential for fertility (2, 4). Studies on GDF-9 null mice have demonstrated arrested follicular development at the primary follicle stage (5). Mouse GDF-9 induces Smad2 phosphorylation and inhibin production in rat diethylstilbestrol treated granulosa cells (6) and in human granulosa-luteal cells (7). The downstream signaling actions of GDF 9 are mediated by the type I receptor, activin receptor-like kinase 5 (ALK5), initiating the subsequent activation of Smad2 and Smad3 (2, 8). GDF 9 uses the BMP type II receptor (BMPRII) as its other signaling receptor (2, 9).

References:

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