HIF-3 alpha Data Sheet

Catalog Number:	RA25075	**Host:**	Rabbit
Product Type:	Affinity Purified	**Species Reactivity:**	Human
Immunogen Sequence:	Synthetic peptide made to an internal portion of the human protein (within residues 650-700). [Swiss-Prot# O75473].	**Format:**	Liquid. Tris-citrate/phosphate buffer, pH 7-8 with

Applications: Western Blot: 1:100-1:200

*Dilutions listed as a recommendation. Optimal dilution should be determined by investigator.

Storage: Store frozen. Aliquot as undiluted antisera and immediately place at -20°C. Antisera may have become trapped in top of vial during shipping. Centrifugation of vial is recommended before opening. Stable for at least 6 months at -20°C. Repeated freeze/thaw cycles compromise the integrity of the antiserum.

Application Notes

May be used in Western analysis where it recognizes a band at ~70 kDa representing HIF-3 alpha. Proteins tested were produced in a rabbit reticulocyte system (Promega's TnT). 10 microliters of a TnT reaction was tested, as this protein tends to be of higher concentration than in native tissue samples. We have not currently been able to detect this protein in tissue extracts. Not tested in any other application. The investigator should determine the optimal working dilution for a specific application.

Western Blot:
1. Perform SDS-PAGE (4-12% MOPS) on samples to be analyzed, loading 30 ug of total protein per lane.
2. Transfer proteins to Nitrocellulose according to the instructions provided by the manufacturer of the transfer apparatus.
3. Rinse membrane with dH2O and then stain the blot using Ponceau S for 1-2 minutes to access the transfer of proteins onto the nitrocellulose membrane. Rinse the blot in water to remove excess stain and mark the lane locations and locations of molecular weight markers using a pencil.
4. Rinse the blot in TBS for approximately 5 minutes.
5. Block the membrane using 5% non-fat dry milk + 1% BSA in TBS, 1 hour at room temperature.
6. Rinse the membrane in dH2O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.
7. Dilute the rabbit anti-LPIN1 primary antibody (NB 110-57150) in blocking buffer and incubate 2 hours at room temperature.
8. Rinse the membrane in dH2O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.
9. Apply the diluted rabbit-IgG HRP-conjugated secondary antibody in blocking buffer (as per manufacturers' instructions) and incubate 1 hour at room temperature.
10. Wash the blot in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturers' instructions (Pierce, ECL).

Note: Tween-20 can be added to the blocking or antibody diultion buffer at a final concentration of 0.05-0.2%, provided it does not interfere with antibody-antigen binding.

FOR RESEARCH USE ONLY

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