NEUROMICS

Lipin 1 (LPIN1) Data Sheet

Catalog Number:	RA25079	**Host:**	Rabbit
Product Type:	Affinity Purified	**Species Reactivity:**	Human, Rat, Mouse,
Immunogen Sequence:	A synthetic peptide to an internal region (within residues 300-400) of the human LPIN1 protein. [Swiss-Prot# Q14693].	**Format:**	Liquid. Concentration 1 mg/ml.

Applications: Immunohistochemistry: 0.5-1.0 ug/ml
Western blot: 0.5-1.0 ug/ml.

*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.

Publications:

1.Hergovich A, et al. Priming-dependent phosphorylation and regulation of the tumor suppressor pVHL by glycogen synthase kinase 3. Mol Cell Biol. 2006 Aug;26(15):5784-96.

2.Naga Prasad SV, Duan Z-H, Gupta MK, et al. Unique MicroRNA Profile in End-stage Heart Failure Indicates Alterations in Specific Cardiovascular Signaling Networks. J Biol Chem 2009;284(40):27487-27499. [PMID: 19641226]

Application Notes

This antibody is useful for Western blot , IF, ICC and IHC-paraffin. In WB a band is seen at ~55 kDa on kidney membrane preps representing GLUT1 protein. Depending on the tissue and any post-translational modifications, this protein can run anywhere between 40-60 kDa.

Immunohistochemistry-paraffin embedded sections

Antigen Retrieval:

Bring slides to a boil in 10 mM sodium citrate buffer pH 6.0 then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench top for 30 minutes.

Staining:

1. Wash sections in dH_2O three times for 5 minutes each.
2. Wash section in wash buffer (1X PBS/0.1% Tween-20 (1X PBST)) for 5 minutes.
3. Block each section with 100-400 µl blocking solution (1X PBST, 5% goat serum) for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 µl primary antibody diluted in 1X PBST, 5% goat serum to each section. Incubate overnight at 4°C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 µl biotinylated secondary antibody, diluted in 1X PBST, 5% goat serum. Incubate 30 minutes at room temperature.

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7. Remove secondary antibody solution and wash sections three times with wash buffer for 5 minutes each.
8. Add 100-400 µl Striptavidin-HRP reagent to each section and incubate for 30 minutes at room temperature.
9. Wash sections three times in wash buffer for 5 minutes each.
10. Add 100-400 µl DAB substrate to each section and monitor staining closely.
11. As soon as the sections develop, immerse slides in dH_2O.
12. Counterstain sections in hematoxylin.
13. Wash sections in dH_2O two times for 5 minutes each.
14. Dehydrate sections.
15. Mount coverslips.

Western Blot:
1. Perform SDS-PAGE (4-12%) on samples to be analyzed, loading 40 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, for 2 hours 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-GLUT1 primary antibody (NB 110-39113) in blocking buffer and incubate overnight at 4 degrees Celcius.
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 manufacturer's 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 manufacturer,s instructions (Pierce's ECL).
Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%, provided it does not interfere with antibody-antigen binding.

Image: LPIN1 staining of mouse pancreas tissue.

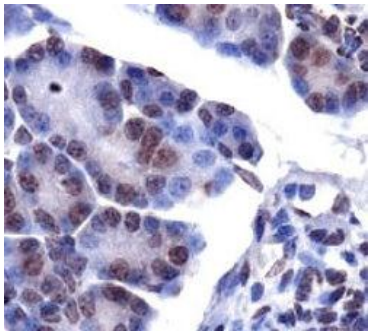
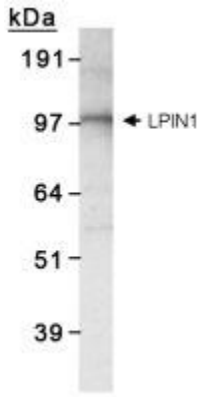


Image: Detection of LPIN1 in 3T3L1 lysate.



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