



Catalog Number:	RA25070	Host:	Rabbit
Product Type:	Affinity Purified	Species Reactivity:	Human, Primate, Rat
Immunogen Sequence:	Synthetic peptide made to an internal portion of the human protein (within residues 1050-1100). [Swiss-Prot# Q9NR96]	Format:	Liquid. Buffered with tris-citrate/phosphate [pH 7-8]. Concentration 1 mg/ml.

Applications: Western blot: 2.0 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.

Application Notes

This antibody is designed for Western blot, where a band is seen ~120 kDa.

Protocol

1. Perform SDS-PAGE (4-12% MOPS) on samples to be analyzed, loading 25 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% NFDM + 1% BSA in TBS + Tween, 1 hour at RT.
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-TLR9 primary antibody in blocking buffer and incubate 1 hour 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 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 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

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www.neuromics.com

Neuromics Antibodies • 5325 West 74th Street, Suite 8 • Edina, MN 55439
phone 866-350-1500 • fax 612-677-3976 • e-mail pshuster@neuromics.com