NEUROMICS

TROY/TNFRSF19 Data Sheet

Catalog Number:	GT15223	**Host:**	Goat
Product Type:	Affinity Purified Antibody	**Species Reactivity:**	Mouse
Immunogen Sequence:	Purified, insect cell line Sf 21-derived, recombinant mouse tumor necrosis factor receptor superfamily 19/TROY (rmTNFRSF19) extracellular domain.	**Format:**	Liquid 1mg/ml Solution in phosphate-buffered saline (PBS) with 5% Trehlose
Applications:	Immunohistochemistry -5-15 µg/mL Western Blot-1 - 2 µg/mL ELISA Capture-0.8 µg/mL, Dilutions listed as a recommendation. Optimal dilution should be determined by investigator.		
Storage:	Antibody can be aliquotted and stored frozen at -20° C to -70° C in a manual defrost freezer for six months without detectable loss of activity. The antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. *Avoid repeated freeze-thaw cycles.*		

Application Notes

Specificity
This antibody has been selected for its ability to recognize mouse TNFRSF19 in the applications listed below.

Western Blot
This antibody can be used at 0.1 - 0.2 µg/mL with the appropriate secondary reagents to detect mouse TNFRSF19. The detection limit for rmTNFRSF19 is approximately 5 ng/lane under non-reducing and reducing conditions.

ELISA Capture
This antibody can be used as a capture antibody in a mouse TROY ELISA in combination with biotinylated, mouse TNFRSF19 affinity purified polyclonal detection antibody (Catalog # :GT15223B). A general protocol is provided on the next page. Using plates coated with 100 µL/well of the capture antibody at 0.8 µg/mL, in combination with 100 µL/well of the detection antibody, an ELISA for sample volumes of 100 µL can be obtained. To arrive at the optimal dose range for this ELISA, set up a two-fold dilution series of the protein standard starting with 4 ng/mL. In this format, less than 2% cross-reactivity with rhTAJ and less than 0.2% cross-reactivity with rmEDAR, rmCD27, rmFas, rmRANK, rmTNF RI and rmTNF RII is observed.

Immunohistochemistry
This antibody will detect TNFRSF19 in cells or tissues. The working dilution is 5 - 15 µg/mL.

Description/Data:
TROY also known as TNFRSF19 (Tumor necrosis factor receptor superfamily, member 19) or TAJ (Toxicity and JNK inducer). TROY is highly expressed during embryonic development. It has been shown to interact with TRAF family members, and to activate JNK signaling pathway when overexpressed in cells. This receptor is capable of inducing apoptosis by a caspase-independent mechanism, and it is thought to play an essential role in embryonic development. It is expressed in GFAP-positive astrocytes of the various regions, such as the cerebral cortex, striatum, and hippocampus. Thus, TROY is expressed in uncommitted precursor cells and astroglial lineage cells, suggesting that TROY plays some roles in the regulation of gliogenesis in the adult CNS.

FOR RESEARCH USE ONLY

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

ELISA Protocol

Solutions Required

- Wash Buffer - 0.05% Tween® 20 in PBS, pH 7.4
- Diluent - 1% BSA in PBS
- Substrate Solution - 1:1 mixture of Color Reagent A (H_20_2) and Color Reagent B (Tetramethylbenzidine) (R&D Systems, Catalog # DY998)
- Stop Solution - 1 M H_2SO_4

Plate Preparation

1. Transfer 100 µL/well of the capture antibody (diluted to the appropriate concentration in PBS, use immediately) to an ELISA plate. Seal plate and incubate overnight at room temperature.
2. Aspirate each well and wash with Wash buffer, repeating the process two times for a total of 3 washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper toweling.
3. Block plates by adding 300 µL of PBS containing 1% BSA, 5% sucrose and 0.05% NaN_3 to each well. Incubate at room temperature for a minimum of 1 hour.
4. Repeat the aspiration/wash as in step 2. The plates are now ready for sample addition. Alternatively, the blocking buffer can be aspirated after step 3 and the plates can be dried under a vacuum. When sealed with desiccant, the plates can be stored at 4° - 8° C for at least 2 months.

Assay Procedure

1. Dilutions of unknowns and standards should be carried out in polypropylene tubes. Add 100 µL of sample or standards in an appropriate diluent, per well. Mix by gently tapping the plate frame for 1 minute. Cover with an adhesive strip and incubate 2 hours at room temperature.
2. Repeat the aspiration/wash as in step 2 of Plate Preparation.
3. Dilute the biotinylated detection antibody with 2% heat-inactivated normal goat serum in the above diluent and mix gently for 1 hour. Add 100 µL to each well. Cover with a new adhesive strip and incubate for 2 hours at room temperature.
4. Repeat the aspiration/wash as in step 2 of Plate Preparation.
5. Add 100 µL streptavidin HRP (R&D Systems, Catalog # DY998, dilute according to the directions on the vial label) to each well. Cover the plate and incubate for 20 minutes at room temperature.
6. Repeat the aspiration/wash as in step 2.
7. Add 100 µL of Substrate Solution to each well. Incubate for 20 - 30 minutes at room temperature. Avoid placing the plate in direct light.
8. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

Calculation of Results

To calculate assay results, average the duplicate readings and subtract the zero standard optical density from the sample optical density. Create a standard curve using data reduction software capable of generating a four parameter (4P-L) curve fit. Alternatively, plot the optical density for the standards versus the concentration of the standards and draw the best curve. The data can be linearized by using log-log paper and regression analysis may be applied to the log transformation. To determine the mouse TROY concentrations for each sample, first find the absorbance value on the y-axis and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the x-axis and read the corresponding mouse TROY concentration. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor. A standard curve should be generated for each set of samples assayed.

Limitations

It is important that the diluents selected for reconstitution and for dilution of the standard reflect the environment of the samples being measured. The diluent suggested in the above protocol may be suitable for most cell culture supernate samples. Validate diluents for specific sample types prior to use. A basic understanding of immunoassay development is required for the successful use of these reagents in immunoassays. The protocol provided is for demonstration purposes only. The type of enzyme and substrate and the concentrations of capture/detection antibodies used can be varied to create an immunoassay with a different sensitivity and dynamic range.

Tween is a registered trademark of ICI Americas.

FOR RESEARCH USE ONLY

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