NEUROMICS

Macrophage Scavenger Receptor I (CD204) Data Sheet

Catalog Number:	RA25074	**Host:**	Rabbit
Product Type:	Affinity Purified	**Species Reactivity:**	Human, Mouse, Rat, Cat, Primate and Bovine
Immunogen Sequence:	Synthetic peptide made to an internal portion of the human protein (within residues 300-400). [Swiss-Prot# P21757].	**Format:**	Liquid. PBS, 30% glycerol with 0.1% Sodium Azide. Concentration : 1.0 mg/ml.
Applications:	Western Blot: 0.5 ug/ml Immunocytochemistry: 1:50 - 1:200 Immunofluorescence: 1:50 - 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

Localization: Membrane; Single-pass type II membrane protein. This antibody is useful for Western Blot, where a band is seen ~50 kDa.

Western Blot Protocol:

1. Perform SDS-PAGE (4-12% MOPS) 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% 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-MSR1 primary antibody (NBP1-100092) 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 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 dilution buffer at a final concentration of 0.05-0.2%, provided it does not interfere with antibody-antigen binding.

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Immunocytochemistry Protocol:
Culture cells to appropriate density in 35mm culture dishes or 6-well plates.

1. Pull off culture medium with and add 10% formalin to the dish. Fix at room temperature for 30 minutes..
2. Take off the formalin and add ice cold methanol (kept in well sealed bottle in -20C). Incubate for 5-10 minutes.
3. Take off methanol and add PBS (You can add 0.1% Tween-20 to PBS used here and all subsequent steps), be sure to not let the specimen dry out. Wash 3 times 10 minutes before proceeding to blocking step.
4. To block nonspecific antibody binding incubate in 10% normal goat serum for a minimum of 1 hr at room temp. Cells can also block overnight at 4C for this step.
5. Add primary antibody at appropriate dilution and incubate at room temp for 2 hrs or overnight at room temp.
6. Remove primary antibody and replace with PBS. Wash 3 x 10 min in PBS.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hr at room temperature
8. Remove antibody and replace with PBS, wash 1 x 10 min in PBS. Add Hoechst 33258 to PBS at 1:25,0000 and incubate for 10 min. Wash a third time with PBS for 10 min (total of 3X10min PBS washes).
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide and parafilmed. Cells can also be coverslipped using Fluoromount. If storing coverslip be sure to seal the edges with clear nail polish.

The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.

Immunohistochemistry Protocol:
Frozen Sections-
1. Snap frozen fresh tissues in liquid nitrogen or isopentane pre-cooled in liquid nitrogen, embedded in OCT compound in cryomolds. Store frozen blocks at -80^{O}C.
2. Cut 4-8 um thick cryostat sections and mount on superfrost plus slides or gelatin coated slides. Store slides at – 80^{O}C until needed.
3. Before staining, warm slides at room temperature for 30 minutes and fix in ice cold acetone for 10 minutes. Air dry for 30 minutes.
4. Wash in PBS.

Paraffin Sections-
1. Deparaffinize sections in xylene, 2x5min.
2. Hydrate with 100% ethanol, 2x3min.
3. Hydrate with 95% ethanol, 1min.
4. Rinse in distilled water.

Tissue Staining-
1. Follow procedure for pretreatment as required.
2. Procedure for Immunoenzyme Staining.
3. Rinse Sections in Washing Buffer (PBS+0.1% Triton X-100, pH 7.4) for 2x2 min.
4. Serum Blocking: incubate sections in normal serum block – species same as secondary antibody(Wash Buffer + 5% Normal Sera). Note: since this protocol uses avidin-biotin detection system, avidin/biotin block may be needed based on tissue type. If you do, the avidin/biotin blocking should be done after normal serum block and before primary antibody incubation.
5. Primary Antibody: incubate sections in primary antibody at appropriate dilution in Wash Buffer for 1 hour at room temperature or overnight at 4C. Note:Do not rinse sections between serum block and primary antibody incubation.
6. Rinse in washing buffer for 3x2 min.
7. Peroxidase Blocking: incubate sections in peroxidase blocking solution for 10 minutes at room temperature.
8. Rinse in washing buffer for 3x2 min.
9. Secondary Antibody: incubate sections in biotinylated secondary antibody at an appropriate dilution in Wash Buffer for 30 minutes at room temperature.
10. Rinse in washing buffer for 3x2 min.
11. Detection: incubate sections in streptavidin-HRP in Wash Buffer for 30 minutes at room temperature.
12. Rinse in washing buffer for 3x2 min.
13. Chromagen/Substrate: incubate sections in peroxidase substrate solution.
14. Rinse in washing buffer for 3x2 min.
15. Rinse in running tap water for 2-5 minutes.
16. Dehydrate through 95% ethanol for 1 minute, 100% ethanol for 2x3min.
17. Clear in xylene for 2x5min.
18. Coverslip with mounting medium **(**i-BRITE Plus-Catalog#: SF40000)

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Peroxidase Block-
0.3% H2O2 in PBS (for Paraffin Sections)
0.3% H2O2 in Methanol (for Frozen Sections)

Avidin/Biotin Block-
0.001% Avidin in PBS
0.001% Biotin in PBS
Block for 10 minutes with first solution, rinse with PBS, block for 10 minutes with second solution.

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Image: MSRI staining (green) in Hela cells at a 1:50 dilution. Nuclei (Blue) are counterstained using Hoechst 33258.

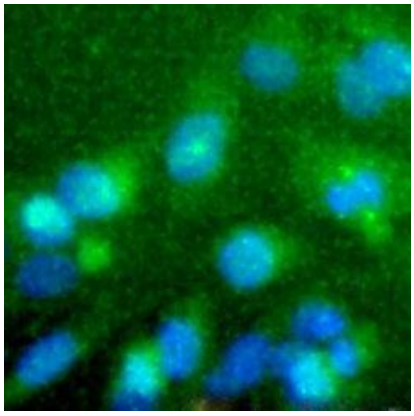
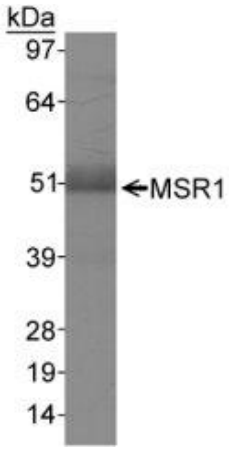


Image: Detection of MSR I in human liver



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