NEUROMICS

CD9/Motility-Related Protein-1 Data Sheet

Catalog Number:	MO20046	**Host:**	Mouse
Ig Class:	IgG_1 Clone: 72F6	**Species Reactivity:**	Human
Immunogen Sequence:	Prokaryotic recombinant protein corresponding to the major extracellular loop of the CD9.	**Format:**	Liquid- tissue culture supernatant containing 15mM sodium azide.

Applications: Immunohistochemistry: 1:75-1:150 (Paraffin wax embedded tissue using the high temperature antigen unmasking technique- 1 mM EDTA, pH 8.0 and 60 minutes primary antibody incubation at 25 °C*, or Frozen tissue using acetone fixation).

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

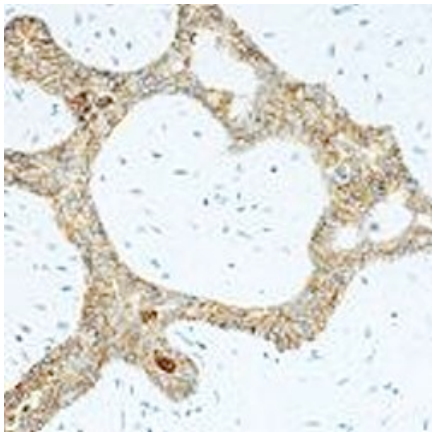
Storage: Antibody can be aliquotted and stored frozen at -20° C to -70° C in a manual defrost freezer for six months without detectable loss of activity. The antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. *Avoid repeated freeze-thaw cycles.*

Application Notes

Positive Controls: Immunohistochemistry: Bowel.
Staining Pattern: Membrane.

Description/Data:
CD9 antigen is a 24 to 27 kD glycoprotein expressed on the surface of developing B lymphocytes, platelets, monocytes, eosinophils, basophils, stimulated T lymphocytes and by neurons and glial cells in the peripheral nervous system. It belongs to a family of membrane proteins termed tetraspanins that transverse the membrane four times. In pre-B cells and platelets, CD9 antigen regulates cell activation aggregation possibly through an association with the integrin CD41/ CD61 (GPIIb/GPIIIa). It also regulates cell motility in a variety of cell lines, and appears to be an important regulator of Schwann cell behaviour in peripheral nerve.

Image: CD9 staining of Human Bowel Section. Paraffin section.



***Immunohistochemistry- High Temperature Antigen Unmasking Technique for Paraffin Wax Embedded Tissue**
Note: Also useful for staining frozen tissue-Acetone fixation recommended.

1. Cut and mount sections on slides coated with a suitable tissue adhesive.
2. Deparaffinize sections and rehydrate to distilled water.

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3. Place sections in 0.5% hydrogen peroxide/methanol for 10 minutes (or use other appropriate endogenous peroxidase blocking procedure). Wash sections in tap water.
4. Heat 1500 mL of the recommended unmasking solution (0.01 M citrate buffer, pH 6.0 (or Epitope Retrieval Solution, RE7113) until boiling in a stainless steel pressure cooker. Cover but do not lock lid.
5. Position slides into metal staining racks (do not place slides close together as uneven staining may occur) and lower into pressure cooker ensuring slides are completely immersed in unmasking solution. Lock lid.
6. When the pressure cooker reaches operating temperature and pressure (after about 5 minutes) start a timer for 1 minute (unless otherwise indicated on the data sheet).
7. When the timer rings, remove pressure cooker from heat source and run under cold water with lid on. DO NOT OPEN LID UNTIL THE INDICATORS SHOW THAT PRESSURE HAS BEEN RELEASED. Open lid, remove slides and place immediately into a bath of tap water.
8. Wash sections in TBS* buffer (pH 7.6) for 1 x 5 minutes.
9. Place sections in diluted normal serum (or RTU Normal Horse Serum) for 10 minutes.
10. Incubate sections with primary antibody. Use Antibody Diluent RE7133 (where available).
11. Wash in TBS buffer for 2 x 5 minutes.
12. Incubate sections in an appropriate biotinylated secondary antibody.
13. Wash in TBS buffer for 2 x 5 minutes.
14. Incubate slides in ABC reagent (or RTU streptavidin/peroxidase complex).
15. Wash in TBS buffer for 2 x 5 minutes.
16. Incubate slides in DAB or other suitable peroxidase substrate.
17. Wash thoroughly in running tap water.
18. Counterstain with hematoxylin (if required), dehydrate and mount.

Solutions
0.01 M CITRATE BUFFER (pH 6.0) or RE7113 (where available).
Add 3.84 g of citric acid (anhydrous) to 1.8 L of distilled water. Adjust to pH 6.0 using concentrated NaOH. Make up to 2 L with distilled water.
1 mM EDTA (pH 8.0) or RE7116 (where available).

Add 0.37 g of EDTA (SIGMA product code E-5134) to 1 litre of distilled water. Adjust pH to 8.0 using 1.0 M NaOH.
20 mM TRIS/ 0.65 mM EDTA/ 0.005% TWEEN (pH 9.0) or RE7119 (where available).
Dissolve 14.4 g Tris (BDH product code 271197K) and 1.44 g EDTA (SIGMA product code E-5134) to 0.55 L of distilled water. Adjust pH to 9.0 with 1 M HCl and add 0.3 mL Tween 20 (SIGMA product code P-1379). Make up to 0.6 L with distilled water. This is a 10x concentrate which should be diluted with distilled water as required (eg 150 mL diluted with 1350 mL of distilled water).

** In most applications, 10 mM phosphate, 0.15 M NaCl, pH 7.6 (PBS) can be used instead of 50 mM Tris, 0.15 M NaCl, pH 7.6 (TBS).*

General References:
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Anton E S, Hadjiargyrou M, Patterson P H, et al.. The Journal of Neuroscience. 15 (1): 584–595 (1995).
Fernvik E, Halldén G, Hed J, et al.. APMIS. 103: 699–706 (1995).
Kaprielian Z, Cho K–O, Hadjiargyrou M, et al.. The Journal of Neuroscience. 15 (1): 562–573 (1995).
Miyake M, Nakano K, Ieki Y, et al.. Cancer Research. 55: 4127–4131 (1995).

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