CD49a PURIFIED HAMSTER ANTI-RAT/MOUSE CD49a (Integrin α_1 chain) ANTIBODY FOR IMMUNOHISTOCHEMISTRY (IHC).

PRODUCT INFORMATION

Catalog Number: **550568** (Was: 75311E), **1 ml**
Clone: Ha31/8
Antibody Isotype: Hamster IgG grp 2
Storage Buffer: Purified IHC diluent containing serum* and 0.09% Sodium Azide.

DESCRIPTION

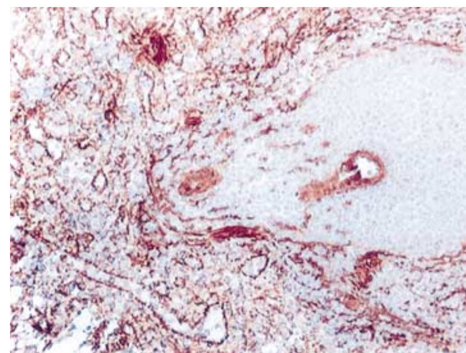
The Ha31/8 antibody reacts with the 180-kDa integrin α_1 chain (CD49a), which is a transmembrane glycoprotein that non-covalently associates with the integrin β_1 subunit (CD29) to form the α_1 β_1 (CD49a/CD29 or VLA-1) complex.[1] VLA-1 has been reported to be expressed on activated T cells, monocytes, smooth muscle cells, and endothelial cells.[2] It is a receptor for collagen and laminin.[3] The Ha31/8 monoclonal antibody is specific for both rat[1] and mouse CD49a.[3,4] It has been reported that Ha31/8 antibody can block VLA-1–mediated binding to collagen.[1] Source of the immunogen was emulsified Lewis rat glomeruli.[1]

PREPARATION AND STORAGE

The antibody is purified from tissue culture supernatant by Protein G affinity chromatography. Store at 4°C.

APPLICATION NOTES

Immunohistochemistry: The Ha31/8 antibody specific for rat/mouse CD49a is tested for immunohistochemical staining of acetone-fixed frozen sections. Tissues tested were rat and mouse spleen and thymus. The antibody stains activated T lymphocytes, monocytes, and endothelial cells. The isotype control recommended for use with this antibody is purified hamster IgG group 2 (Cat. No. 550345/74531E). For optimal indirect immunohistochemical staining, the Ha31/8 antibody should be titrated (1-10 to 1-50 dilution) and visualized via a three-step staining procedure in combination with, biotin conjugated anti-hamster IgG cocktail (Cat. No. 550335/74442E) as the secondary antibody and Streptravidin-HRP (please inquire) together with the DAB detection system (please inquire). A detailed protocol of the immunohistochemical procedure is enclosed. ***The clone Ha31/8 is not recommended for formalin-fixed paraffin embedded sections.***



Immunohistochemical staining of CD49a positive cells: Frozen sections of normal rat spleen were reacted with the anti-CD49a antibody. Cells expressing CD49a can be identified by the brown labeling of their cell membranes. Amplification 20X.

OTHER APPLICATIONS

The Ha31/8 antibody has been tested by immunofluorescent staining for flow cytometric analysis and is available in different fluorochrome conjugated formats. Please contact Technical Service Department for further details.

REFERENCES:

1. Mendrick, D.L., D.M. Kelly, S.S. duMont, and D.J. Sandstrom. 1995. Glomerular epithelial and mesangial cells differentially modulate the binding specificities of VLA-1 and VLA-2. *Lab. Invest.* 72: 367 - 375.
2. Hemler, M.E. 1990. VLA proteins in the integrin family: structures, functions, and their role in leukocytes. *Annu. Rev. Immunol.* 8: 365 – 400.
3. Miyake, S., T. Sakurai, K. Okumura, and H. Yagita. 1994. Identification of collagen and laminin receptor integrins on murine T lymphocytes. *Eur. J. Immunol.* 24: 2000 - 2005.

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Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.

* Source of all serum is from the United States

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PROTOCOL FOR IMMUNOHISTOCHEMICAL STAINING OF FROZEN SECTIONS

1. Preparation of Frozen Tissues for sectioning

Materials needed:

2-methylbutane (isopentane)
Liquid nitrogen
Dry ice
Peel-Away® base molds
Frozen tissue matrix (OCT® or Cryomatrix®)
Long forceps
Necropsy tools
Superfrost Plus slides.

1. Label base mold and partially fill the mold with frozen tissue matrix.
2. Sacrifice animal by prescribed and approved euthanasia techniques.
3. Remove desired tissues and place in pre-labeled base molds filled with frozen tissue matrix. Try to arrange tissue in matrix near the bottom so tissue is easily exposed when sections are cut.
4. Plunge base mold with tissue in frozen tissue matrix into 2-methylbutane prechilled in a dewar of liquid nitrogen until the block ALMOST solidifies (30 seconds). NOTE: If the block is left in too long, it may crack.
5. Remove tissue block from 2-methylbutane and place blocked tissues on dry ice. (Tissues may be stored in the base molds or transferred to plastic bags.)
6. Store frozen tissue blocks in -70°C freezer until sectioning.
7. For sectioning, attach the frozen tissue block on the cryostat chuck. Allow tisue block to equilibrate to the cryostat temperature (-20°C) before cutting sections. Routine sections are cut at 5 microns and picked up onto slide.
8. Dry at room temperature till the sections are firmly adhered to the slide.
9. Fix sections in cold acetone (-20°C) for 2 min. Dry fixed slides completely (usually 1 hour at room temperature). Store in a -70°C freezer until use.

2. Standard Immunohistochemical Staining Procedure For Frozen Sections

Please read entire procedure before staining sections. Perform all incubations in a humid chamber and do not allow sections to dry out. Isotype and system controls should also be run and must be matched to the isotype of each primary antibody to be tested.

Materials needed:

Phosphate Buffered Saline (PBS)
Blocking buffer (please inquire)
Antibody diluent for IHC (Cat. No. 559148/70991A)
Streptravidin/ HRP (please inquire)

1. Remove frozen slides from freezer and allow to come to RT.
2. Rinse slides 2-3 times in PBS to remove frozen mounting media.
3. Apply the blocking buffer and incubate for 10 min.
4. Rinse slides in 3 changes of PBS, 2 min each.
5. Dilute primary antibody in antibody diluent for IHC (Cat. No. 559148/70991A). Apply to cover tissue sections on slide and incubate 1 hr at RT in a humid chamber.
6. Rinse slides in 3 changes of PBS, 2 min each.
7. Dilute biotinylated secondary antibody in antibody diluent (Cat. No. 559148/70991A). Apply onto tissue sections and allow to incubate at RT for 30 min.
8. Rinse slides in 3 changes of PBS, 2 min each.
9. Apply pre-diluted Strepavidin/HRP to each slide and incubate at RT 30 min.
10. Rinse slides in 3 changes of PBS, 2 min each.
11. Prepare DAB according to manufacturer's specifications. SAFETY NOTE: DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
12. Drain slides and add DAB solution to the sections. Allow to incubate 5 min or less till the desired color intensity is reached.
13. Drain excess DAB on paper towel and Rinse slides well in water 3 times.
14. Counterstain slides:
    a. Dip twice in Hematoxylin.
    b. Rinse thoroughly in water.
    c. Dip twice in Bluing Reagent or dilute ammonia water.
    d. Rinse thoroughly in water.
15. Dehydrate through 4 changes of increasing grades of alcohol to 100%, clear in 3-4 changes of xylene (or xylene substitute) and coverslip.