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ProductInformation

Monoclonal Anti-Integrin α2 (CD49b, VLA-2α)
Clone A2-IIE10
Purified Mouse Immunoglobulin

Product Number **I 2659**

Product Description

Monoclonal Anti-Integrin α2 (mouse IgG isotype) is derived from the A2-IIE10 hybridoma produced by the fusion of P3XAg8.653 mouse myeloma cells and splenocytes from a RBF/DnJ mouse immunized with a A549 human lung carcinoma cell line.[1] The antibody is purified from ascites fluid using protein G.

Monoclonal Anti-Integrin α2 is specific for human integrin α2 (160 kDa). It does not cross-react with mouse integrin α2, and other species cross reactivity has not determined. The antibody may be used for immunocytochemistry and blocking cellular adhesion to collagen.

Integrins are important extracellular matrix (ECM) receptor proteins located on cell surfaces. They are heterodimers composed of an α and β transmembrane glycoprotein subunit. Approx. 22 different integrins (different α and β subunit combinations) are found in nature. Integrins are generally present in high concentrations at the cell surface, but, unlike most other cell-surface receptors, they bind ligands with very low affinity. Due to their weak individual binding, integrins need to cluster and bind in groups in order to effectively bind the ECM. Integrins bind many different ligands including laminin. Each integrin is made up of a large N-terminal extracellular domain that binds the ECM ligand and a small C-terminal cytoplasmic domain that mediates interaction with the actin cytoskeleton and signaling function.[2]

Integrin α-2 is a receptor for collagen and laminin[3]. α-2 is expressed in platelets and monocytes and is found in many places in normal tissue.[4]

Reagent

The antibody is supplied as ~ 200 µg of purified mouse immunoglobulin in 200 µL of 0.1 M Tris-glycine, pH 7.4, and 0.15 M sodium chloride.

Storage/Stability

Store at 0 °C to –20 °C. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

Procedures

Immunocytochemistry

1. Plate approximately 200 µL of a cell suspension into each well of a slide. Incubate 24 hours in a 37 °C CO_2 incubator.
2. Wash the cells 3 X for 5 min with PBS. Do not shake cells.
3. Add fixative (ice-cold 95% ethanol, 5% acetic acid) for 1 min at room temperature.
4. Wash the cells with PBS, 2 X for 30 min with gentle agitation. Do not shake cells.
5. Add 400 µL PBS containing 0.8% BSA and incubate 30 minutes at room temperature.
6. Wash cells 2X with PBS for 30 min.
7. Incubate the cells with 10 µg/mL of Anti-Integrin α2 in PBS and incubate overnight at 2-8 °C.
8. Wash the cells 2 X with PBS for 30 min.
9. Incubate the cells with a 1:150 dilution of anti-mouse IgG conjugated with FITC (Sigma Product No. F5262) in PBS for 2 hr. at room temperature in the dark.
10. Wash the cells 3 X with PBS for 30 min in the dark.
11. Mount coverslips with gel mount and allow gel mount to dry in the dark.
12. Examine the cells under a fluorescent microscope.

Adhesion Blockade

1. Precoat wells with 5 µg/mL collagen in DMEM for 1 hour at 2-8 °C.
2. Block with 0.1% BSA for 45 min. at 37 °C.
3. Detach cells using 2 mM EDTA in Ca^{2+} and Mg^{2+} free PBS.
4. Resuspend cells to a concentration of approximately 2 x 10^4 cells/mL in media.
5. Incubate cells with different concentrations of Anti-Integrin α2 (1-10 µg/mL) for 30 min at 2-8 °C.
6. Plate cells and incubate 30 min. at 37 °C.
7. Wash plates 3 X with media, gently. Count unattached cells.
8. Add 1 mL dissociation fluid and count attached cells.

Product Profile

A working concentration of 10-15 µg/ml is recommended for immunocytochemistry using cold-ethanol/acetic acid (95:5) fixed cells.

Note: In order to obtain best results and assay sensitivity in different techniques and preparations we recommend determining optimal working dilutions by titration.

References

1. Lee, R.T., et al., Cir. Res., **76,** 209 (1995).
2. Chan, B.M., et al., Cell, **68,** 1051 (1992).
3. Bergelson, J.M., et al., Cell Adhes. Commun., **2**, 455 (1994).
4. Takada, et al., J Cell Biol., **109**, 397 (1989).

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