

Product Information

MONOCLONAL ANTI-HUMAN INTEGRIN α 2 (CD49b, VLA-2), CLONE A2-IIE10 Purified Mouse Immunoglobulin

Product Number **I 6403**

Product Description

Monoclonal Anti-Human Integrin α 2 (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 line¹. The antibody is purified from ascites fluid using protein G.

Monoclonal Anti-Human Integrin α 2 is specific for human integrin α 2 (160 kDa). It does not cross-react with mouse integrin α 2, 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 alpha and a beta transmembrane glycoprotein subunit. Around twenty-two different integrins (different alpha/ beta 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 alpha-2 is a receptor for collagen and laminin³. Alpha-2 is expressed in platelets and monocytes and is found in many places in normal tissue⁴.

Reagents

Monoclonal Anti-Human Integrin α 2 is supplied as purified mouse immunoglobulin in 0.1 M Tris-glycine, pH 7.4, containing 0.15 M NaCl and 0.05% sodium azide.

Precautions and Disclaimer

Due to the sodium azide, content a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous and safe handling practices.

Storage/Stability

For continuous use, store at 2 °C -8 °C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing is not recommended. Storage in "frost-free" freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

Procedures

Immunocytochemistry

1. Plate approximately 200 μ l of a cell suspension into each well of a slide. Incubate 24 hours in a 37°C. CO₂ 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 μ l 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 μ g/ml of Anti-Human Integrin α 2 in PBS and incubate overnight at 4°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 4°C.
2. Block with 0.1% BSA for 45 min. at 37°C.
3. Detach cells using 2 mM EDTA in Ca²⁺ and Mg²⁺ free PBS.
4. Resuspend cells to a concentration of approximately 2 x 10⁴ cells/ml in media.
5. Incubate cells with different concentrations of Anti-Human Integrin α2 for 30 min. at 4°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, count attached cells.

Product Profile

A working concentration of 10-15 µg/ml is recommended for immunocytochemistry using cold-ethanol-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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