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ProductInformation

MONOCLONAL ANTI-HUMAN INTEGRIN α1 (CD49a, VLA-1α), CLONE 5E8D9
Purified Mouse Immunoglobulin

Product Number **I 6278**

Product Description

Monoclonal Anti-Human Integrin α1 (mouse IgG2a isotype) is derived from the 5E8D9 hybridoma produced by the fusion of SP2/0 mouse myeloma cells and splenocytes from a Balb/c mouse immunized with purified human intergrin α1[1]. The antibody is purified from ascites fluid using protein A.

Monoclonal Anti-Human Integrin α1 is specific for human Integrin α1 (150 kDa). Species cross-reactivity is 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 hetrodimers 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]

Alpha1 integrin along with alpha2, alphaL and alphaM has a unique inserted domain. Integrin alpha1 is a receptor for laminin and collagen[3]. The alpha1 subunit is also known as CD49a

Reagents

Monoclonal Anti-Human Integrin α1 is supplied as purified mouse immunoglobulin in 0.1 M Tris-glycine, pH 7.4, 0.15 M NaCl, 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 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× 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× for 15 min. with gentle agitation. Do not shake cells.
5. Add 400 µl PBS containing 0.08% BSA and incubate 30 minutes at room temperature.
6. Wash cells with PBS for 15 min.
7. Incubate the cells with 10 µg/ml of Anti-Human Integrin α1 in PBS containing 0.08% BSA and incubate overnight at 4°C.
8. Wash the cells 2× with PBS for 5 min.
9. Incubate the cells with a 1:150 dilution of anti-mouse IgG conjugated with FITC (Product No. F 5262) in PBS for 1 hr. at room temperature in the dark.
10. Wash the cells 3× with PBS for 15 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 24 well plate with 20 μg/ml collagen in DMEM for 1 hour at 37 °C (leave 6 wells uncoated for controls).
2. Block with 1 ml of 1% BSA in Ca^{2+}/Mg^{2+} free PBS (CMF-PBS) for 1 hour at 37 °C.
3. Wash 2× with CMF-PBS.
4. Prepare a 2 x 10^4 cells/ml in media suspension in media.
5. Incubate cells with different concentrations of Anti-Human Integrin α1 for 45 min.
6. Add 1ml cell suspension to each well and incubate 1 hour at 37 °C (negative control: no antibody added to non-coated wells; positive control: no antibody added to coated wells).
7. Wash plates 3× with media, gently.
8. Examine cells under microscope

Product Profile

A working concentration of 10 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 test.

References

1. Luque, A., et al., FEBS Lett., **346**, 278 (1994).
2. Chan, B.M., et al,Cell, **68**, 1051 (1992).
3. Gardner, et al., Dev. Biol., **301**, 301 (1996).

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