3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

MONOCLONAL ANTI-HUMAN INTEGRIN αV (CD51), CLONE P2W7
Mouse Ascites Fluid

Product Number **I 6778**

Product Description

Monoclonal Anti-Human Integrin αV (mouse IgG1 isotype) is derived from the P2W7 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from a mouse immunized with an ocular melanoma cell line expressing high levels of human integrin $\alpha_V\beta_1$.

Monoclonal Anti-Human Integrin αV is specific for human integrin αV (160 kDa). The antibody may be used for immunoblotting, immunoprecipitation and immunohistochemistry (frozen tissue).

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.[1]

A subset of integrins contains the vitronectin α subunit receptor, α_V. Alpha V integrins serve as receptors for extracellular matrix proteins and can interact with ligands through the arginine-glycine-aspartic acid (RGD) recognition motif. The alpha V subunit can be associated with different bete subunits, including β_1, β_3, β_5, β_6, and β_8.[2, 3]

Reagents

Monoclonal Anti-Human Integrin αV is supplied as mouse ascites fluid.

Storage/Stability

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

Procedure

Immunoprecipitation

1. Dilute the cell lysate before beginning the immunoprecipitation to roughly 1 mg/ml total cell protein in a microcentrifuge tube with PBS (Sigma Product No. P 3813).
2. Add 7.5 µl of Anti-Human Integrin αV (I 6778) to 1mg cell lysate.
3. Gently rock the reaction mixture at 4 °C overnight.
4. Capture the immunocomplex by adding 100 µl of a washed (in PBS) 1:1 slurry of Protein G-Agarose beads (50 µl packed beads) (Sigma Product No. P 2294).
5. Gently rock reaction mixture at 4 °C for 2 hours.
6. Collect the agarose beads by pulsing (5 seconds in the microcentrifuge at 14,000 x g), and drain off the supernatant. Wash the beads 3 times with either ice cold cell lysis buffer or PBS.
7. Resuspend the agarose beads in 50 µl 2X Laemmli sample buffer. The agarose beads can be frozen for later use.
8. Suspend the agarose beads in Laemmli sample buffer and boil for 5 minutes. Pellet the beads using a microcentrifuge pulse. SDS-PAGE and subsequent immunoblotting analysis may be performed on a sample of the supernatant.

Lysis Buffer:
50 mM Tris-HCl, pH 7.4, containing 1% NP-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EGTA, 1 mM PMSF, 1 µg/ml each aprotinin, leupeptin, pepstatin, 1 mM Na_3VO_4, and 1 mM NaF.

Product Profile

A working antibody dilution of 1:600 is recommended for immunoblotting using CEF (chick embryo fibroblast) cells, anti-goat IgG-peroxidase conjugate, and chemiluminescent detection.

For immunoprecipitation, 7.5 µl is recommended to immunoprecipitate Integrin αV from 1 mg of a CEF RIPA cell lysate.

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

References

1. Chan, B. M., et al., Cell, **68**, 1051 (1992).
2. Bossy, B., and Reichardt, L.F., Biochem., **29,** 10191 (1990).
3. Rajaraman, R., Exp. Cell Res., **205**, 25 (1993).

kaa/lpg/nw 02/02