

User Manual

Beta Integrin Screening Investigator Kit

ECM440

FOR RESEARCH USE ONLY

Not for use in diagnostic procedures. Not for human or animal consumption.

Product Overview

Mouse Anti-Human Integrin beta1 (Activating) Monoclonal Antibody

Component: MAB1951Z-20

Quantity: 20 µg (Note: This antibody is available in a larger size, MAB1951Z)

Concentration: 1 mg/mL

Specificity: Human integrin beta1. The epitope recognized is Ca²⁺ dependent.

Immunogen: Human IL-1beta activated endothelial cells

Isotype: IgG1

Clone: P4G11

Applications:

- IH: acetone or 2% paraformaldehyde fixed frozen tissues or 4% PFA paraffin-embedded (Qin, et al. 2003: 1:500, citrate HIER treated); traditional formalin fixation not recommended.
- Ca²⁺ required for binding. Ca²⁺ must be used in blocking, wash, and antibody incubation buffers.
- IC: 2 µg/mL in the presence of calcium
- FC: 2-5 µg /10⁶ cells, in the presence of Ca²⁺
- IP: 5 µg/0.4 mL lysate (0.5% Triton™ PBS/ Ca²⁺)
- Stimulation of cells: 2-10 µg/mL per 10⁶ cells Binding buffer must contain 1 mM Ca²⁺
- ELISA: Optimal working dilutions must be determined by the end user

Format: Purified immunoglobulin by protein A chromatography

Presentation: Liquid in 0.02 M PBS pH 7.6, 0.25 M NaCl

Mouse Anti-Human Integrin beta2 Monoclonal Antibody

Component: MAB1962Z-20

Quantity: 20 µg (Note: This antibody is available in a larger size, MAB1962Z)

Concentration: 1 mg/mL

Specificity: Integrin beta2 on hematopoietic cells; not present on platelets, endothelial cells, epithelial cells or fibroblasts

Isotype: IgG3

Clone: P4H9-A11

Applications:

- IC: 1:1000
- Aggregation inhibition assays: EBV-transformed B lymphocytes or hematopoietic cells. For extensive dilution, protein-containing or another stabilizing medium should be used.
- Optimal working dilutions must be determined by the end user.

Format: Purified immunoglobulin by protein A chromatography

Presentation: Liquid in 0.02 M PBS, pH 7.6, 0.25 M NaCl

Mouse Anti-Human Integrin beta3 (CD61) Monoclonal Antibody

Component: MAB1957Z-20

Quantity: 20 µg (Note: This antibody is available in a larger size, MAB1957Z)

Concentration: 1 mg/mL

Specificity: Reacts with human integrin beta3. The MW of integrin beta3 is 105 kDa reduced and 90 kDa unreduced. Ligands are fibronectin, fibrinogen, von Willebrand factor, vitronectin and thrombospondin. Residues 237-248 of integrin beta3 are critical in adhesive protein binding. Found on endothelial cells, some B cells, monocytes/macrophages, and tumor cells. The monocytoid cell line U937 is highly positive.

Immunogen: Normal blood mononuclear cells activated by mixed lymphocyte reaction and cultured for 10 days in medium containing IL-2.

Isotype: IgM (original paper claimed IgG2a_κ)

Clone: 25E11

Applications:

- IC: acetone fixation required
- Inhibits platelet aggregation: 10 µg/mL
- Optimal working dilutions must be determined by end user

Format: Purified immunoglobulin by protein A chromatography

Presentation: Liquid in 0.02 M PBS pH 7.6, 0.25 M NaCl

Mouse Anti-Human Integrin beta4 (CD104) Monoclonal Antibody

Component: MAB2060-20

Quantity: 20 µg (Note: This antibody is available in a larger size, MAB2060)

Concentration: 1 mg/mL

Specificity: Human beta4 integrin. Integrin alpha6/beta4 is a receptor for laminin. It plays a critical structural role in the hemidesmosome of epithelial cells.

Immunogen: Human squamous cell carcinoma cells (SCC280)

Isotype: IgG2a_κ

Clone: ASC-9

Applications:

- IH: acetone-fixed frozen human epithelial tissues including tongue, foreskin and ovary. Not for use on formalin-fixed tissue.
- FC: human squamous cell carcinoma (SCC9), ovarian carcinoma (SKOV-3) and umbilical vein endothelium (HUVEC). Does not stain monocytes, neutrophils, lymphocytes, T cells, B cells or platelets.
- IP: precipitates SDS-PAGE bands of 66, 76, 120, 135 and 200 kDa (reduced) or 66, 110, 125, 130 and 190 kDa (non-reduced) from surface-biotinylated SCC9 cells.
- Inhibition of cell attachment inhibits SCC9 cell adhesion to human and EHS laminin, but not to laminin GD-2 peptide, type IV collagen or fibronectin. Does not inhibit SKOV-3 cell adhesion to laminin, fibronectin or type IV collagen.
- Optimal working dilutions must be determined by end user.

Format: Purified immunoglobulin by protein A chromatography.

Presentation: Liquid in 0.02 M PBS pH 7.6, 0.25 M NaCl containing 0.1% sodium azide

Rabbit Anti-Integrin Beta5 Polyclonal Antibody

Component: AB1926-20

Quantity: 20 µL (Note: This antibody is available in a larger size, AB1926)

Concentration: 1 mg/mL

Specificity: Integrin beta5. No cross-reactivity to integrins beta1 or beta3. Recognizes an epitope on the cytoplasmic domain of the protein with an apparent MW of 88 kDa.

Immunogen: (KTFNKFNKSYNGTVD) Synthetic peptide derived from the C-terminus (cytoplasmic domain) of human integrin beta5 subunit. (Note: A lysine (K) residue was added to the original peptide sequence to facilitate coupling to the carrier protein.)

Applications:

- WB: 1:100
- IH(P): acetone-fixed frozen tissues at 1:40-80 or paraffin-embedded at 1:200
- IC: 1:100
- IP 5 µL of antibody is sufficient to precipitate integrin complex alphaV/beta5 from 2.5×10^6 cells
- Optimal working dilutions must be determined by end user

Format: Purified immunoglobulin by protein A chromatography

Species Reactivities: Human, mouse, rat, and hamster

Format: Rabbit serum

Presentation: Liquid with 0.05% sodium azide

Materials Provided

Kit Components

- Integrin beta1 Monoclonal Antibody (MAB1951Z-20) - 1 tube, 20 µg
- Integrin beta2 Mouse Monoclonal Antibody (MAB1962Z-20) - 1 tube, 20 µg
- Integrin beta3 Mouse Monoclonal Antibody (MAB1957Z-20) - 1 tube, 20 µg
- Integrin beta4 Mouse Monoclonal Antibody (MAB2060-20) - 1 tube, 20 µg
- Integrin beta5 Rabbit Polyclonal Antibody (AB1926-20) - 1 tube, 20 µL

Warnings and Precautions

Wear gloves when using this product. Avoid skin contact or ingestion of all reagents and chemicals used in this protocol.

Note: Due to the small volumes contained within the kit, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge before opening to dislodge any liquid entrapped in the container's cap during shipment.

Storage and Stability

Store antibodies at $-20\text{ }^{\circ}\text{C}$.

References

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