

Anti-Integrin beta 4/ITGB4 Antibody Picoband™ (monoclonal, 7G10D2)

Catalog # ABO16569

Product Information

Application	WB, IF, ICC, FC
Primary Accession	P16144
Host	Mouse
Isotype	Mouse IgG2b
Reactivity	Human
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-Integrin beta 4/ITGB4 Antibody Picoband™ (monoclonal, 7G10D2) . Tested in Flow Cytometry, IF, ICC, WB applications. This antibody reacts with Human.
Reconstitution	Adding 0.2 ml of distilled water will yield a concentration of 500 µg/ml.

Additional Information

Gene ID	3691
Other Names	Integrin beta-4, GP150, CD104, ITGB4
Calculated MW	202167
Application Details	Western blot, 0.25-0.5 µg/ml, Human Immunocytochemistry/Immunofluorescence, 5 µg/ml, Human Flow Cytometry, 1-3 µg/1x10 ⁶ cells, Human
Source	Eukaryota
Contents	Each vial contains 4 mg Trehalose, 0.9 mg NaCl and 0.2 mg Na ₂ HPO ₄ .
Clone Names Immunogen	Clone: 7G10D2 E.coli-derived human Integrin beta 4/ITGB4 recombinant protein (Position: R29-K431).
Purification	Immunogen affinity purified.
Storage	At -20°C for one year from date of receipt. After reconstitution, at 4°C for one month. It can also be aliquotted and stored frozen at -20°C for six months. Avoid repeated freezing and thawing.

Protein Information

Name	ITGB4
Function	Integrin alpha-6/beta-4 is a receptor for laminin. Plays a critical structural role in the hemidesmosome of epithelial cells. Is required for the regulation of keratinocyte polarity and motility. ITGA6:ITGB4 binds to NRG1 (via EGF domain) and this binding is essential for NRG1-ERBB signaling (PubMed: 20682778). ITGA6:ITGB4 binds to IGF1 and this binding is essential for IGF1 signaling (PubMed: 22351760). ITGA6:ITGB4 binds to IGF2 and this binding is essential for IGF2 signaling (PubMed: 28873464).
Cellular Location	Cell membrane; Single-pass type I membrane protein. Cell membrane; Lipid-anchor. Cell junction, hemidesmosome. Note=Colocalizes with DST at the leading edge of migrating keratinocytes
Tissue Location	Integrin alpha-6/beta-4 is predominantly expressed by epithelia. Isoform beta-4D is also expressed in colon and placenta Isoform beta-4E is also expressed in epidermis, lung, duodenum, heart, spleen and stomach

Background

ITGB4(Integrin, beta-4), also known as CD104 (Cluster of Differentiation 104), is a human gene. The gene encodes the integrin beta 4 subunits, a receptor for the laminins. This subunit tends to associate with alpha 6 subunits and is likely to play a pivotal role in the biology of invasive carcinoma. The ITGB4 gene is mapped on 17q25.1. Using expression profiling, Yang et al. found that ITGB4 was upregulated 6-fold by ZKSCAN3 in transfected human colon cancer cells compared with parental cells. They confirmed that ZKSCAN3 bound the promoter of ITGB4 in vitro and in vivo. ITGB4 knockdown by short hairpin RNA countered ZKSCAN3-augmented anchorage-independent colony formation in the colon cancer cell lines. The integrin beta-4 subunit is characterized by an unusually long cytoplasmic domain that harbors 4 fibronectin type III (FNIII) repeats, residing in 2 pairs separated by a connecting segment. Vidal et al. found compound heterozygosity for mutations in the ITGB4 gene in an infant with junctional epidermolysis bullosa associated with pyloric atresia.

Images

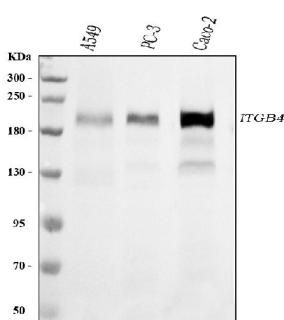


Figure 1. Western blot analysis of ITGB4 using anti-ITGB4 antibody (M01015-2).

Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions.

Lane 1: human A549 whole cell lysates,
Lane 2: human PC-3 whole cell lysates,
Lane 3: human CACO-2 whole cell lysates.

After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with mouse anti-ITGB4 antigen affinity purified monoclonal antibody (Catalog # M01015-2) at 0.5 µg/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-mouse IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1001) with Tanon 5200 system. A specific band was detected for ITGB4 at approximately 210 kDa. The expected band size for ITGB4 is at 202 kDa.

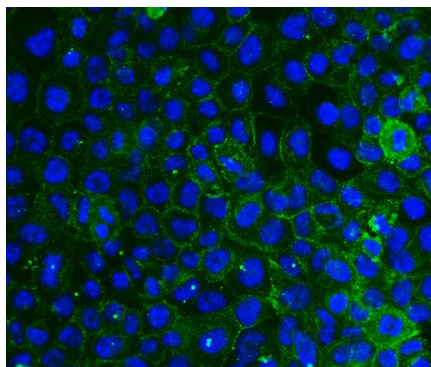


Figure 2. IF analysis of ITGB4 using anti-ITGB4 antibody (M01015-2).

ITGB4 was detected in an immunocytochemical section of A431 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5 µg/mL mouse anti-ITGB4 Antibody (M01015-2) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Mouse IgG (BA1126) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

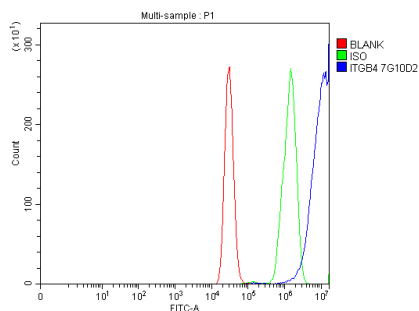


Figure 3. Flow Cytometry analysis of MCF-7 cells using anti-ITGB4 antibody (M01015-2).

Overlay histogram showing MCF-7 cells stained with M01015-2 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-ITGB4 Antibody (M01015-2, 1 µg/1x10⁶ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x10⁶ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x10⁶) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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