

Anti-Integrin beta 4/ITGB4 Antibody Picoband™

Catalog Number: PB9007

About ITGB4

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.

Overview

Product Name	Anti-Integrin beta 4/ITGB4 Antibody Picoband™
Reactive Species	Human
Description	Boster Bio Anti-Integrin beta 4/ITGB4 Antibody Picoband™ catalog # PB9007. Tested in Flow Cytometry, IF, IHC, IHC-F, ICC, WB applications. This antibody reacts with Human.
Application	Flow Cytometry, IF, IHC, IHC-F, ICC, WB
Clonality	Polyclonal
Formulation	Each vial contains 5mg BSA, 0.9mg NaCl, 0.2mg Na2HPO4, 0.05mg NaN3.
Storage Instructions	Store 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 freeze-thaw cycles.
Host	Rabbit
Uniprot ID	P16144

Technical Details

Immunogen	E.coli-derived human Integrin beta 4 recombinant protein (Position: N28-A266). Human Integrin beta 4 shares 88% and 86% amino acid (aa) sequences identity with mouse and rat Integrin beta 4, respectively.
Predicted Reactive Species	Bovine, Chicken, Horse, Monkey, Rabbit
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Rabbit IgG (EK1002) for Western blot, and HRP Conjugated anti-Rabbit IgG Super Vision Assay Kit (SV0002-1) for IHC(P), IHC(F) and ICC.
Cross Reactivity	No cross-reactivity with other proteins

Isotype	Rabbit IgG
Form	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.
Purification	Immunogen affinity purified.
Suggested Dilutions	<p>Dilute the sample so that the expected range of concentrations fall within the detection range of this kit.</p> <p>If the expected range of concentration is unknown, a pilot test should be conducted to decide the optimal dilution ratio for your samples.</p> <p>Some PubMed article(s) citing the expression level of this target are as follows:</p> <p>Boster Bio's internal QC testing used:</p> <p>Western blot, 0.1-0.5ug/ml, Human</p> <p>Immunohistochemistry (Paraffin-embedded Section), 0.5-1ug/ml, Human, By Heat</p> <p>Immunofluorescence, 2ug/ml, Human</p> <p>Immunohistochemistry (Frozen Section), 0.5-1ug/ml, Human</p> <p>Immunocytochemistry, 0.5-1ug/ml, Human</p> <p>Flow Cytometry, 1-3ug/1x10⁶ cells, Human</p>

Anti-Integrin beta 4/ITGB4 Antibody Picoband™ (PB9007) Images

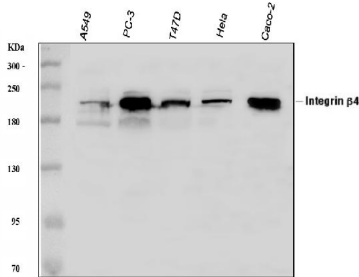


Figure 1. Western blot analysis of ITGB4 using anti-ITGB4 antibody (PB9007).

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 T-47D whole cell lysates,

Lane 4: human Hela whole cell lysates,

Lane 5: 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 rabbit anti-

ITGB4 antigen affinity purified polyclonal antibody (Catalog # PB9007) at 0.5 ug/mL overnight at 4°C, then washed with

TBS-0.1%Tween 3 times with 5 minutes each and probed

with a goat anti-rabbit IgG-HRP secondary antibody at a

dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit

(Catalog # EK1002) 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.

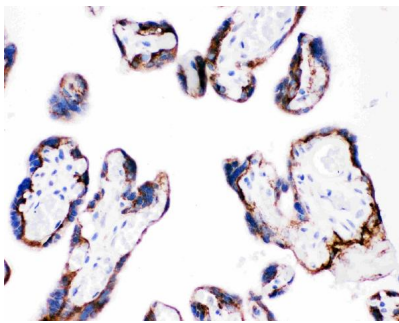


Figure 2. IHC analysis of ITGB4 using anti-ITGB4 antibody (PB9007).

ITGB4 was detected in paraffin-embedded section of human placenta tissues. Heat mediated antigen retrieval was

performed in citrate buffer (pH6, epitope retrieval solution)

for 20 mins. The tissue section was blocked with 10% goat

serum. The tissue section was then incubated with 1ug/ml

rabbit anti-ITGB4 Antibody (PB9007) overnight at 4°C.

Biotinylated goat anti-rabbit IgG was used as secondary

antibody and incubated for 30 minutes at 37°C. The tissue

section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

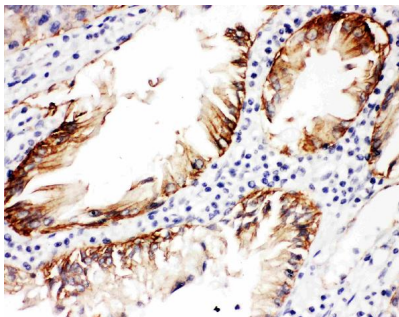


Figure 3. IHC analysis of ITGB4 using anti-ITGB4 antibody (PB9007).

ITGB4 was detected in paraffin-embedded section of human intestinal cancer tissues. Heat mediated antigen retrieval

was performed in citrate buffer (pH6, epitope retrieval

solution) for 20 mins. The tissue section was blocked with

10% goat serum. The tissue section was then incubated with

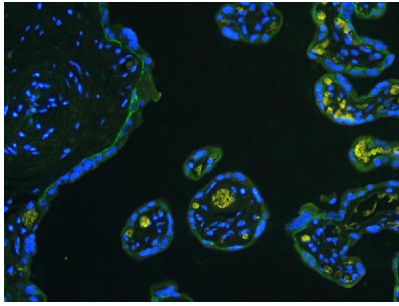
1ug/ml rabbit anti-ITGB4 Antibody (PB9007) overnight at

4°C. Biotinylated goat anti-rabbit IgG was used as secondary

antibody and incubated for 30 minutes at 37°C. The tissue

section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

Figure 4. IF analysis of ITGB4 using anti-ITGB4 antibody



(PB9007)

ITGB4 was detected in paraffin-embedded section of human placenta tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/mL rabbit anti-ITGB4 Antibody (PB9007) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Rabbit IgG (BA1127) 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.

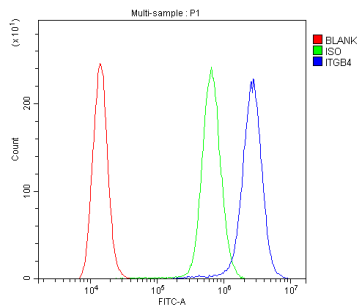


Figure 5. Flow Cytometry analysis of A431 cells using anti-ITGB4 antibody (PB9007).

Overlay histogram showing A431 cells stained with PB9007 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-ITGB4 Antibody (PB9007, 1ug/1x10⁶ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10ug/1x10⁶ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1ug/1x10⁶) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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