abcam

Product datasheet

Anti-Integrin beta 1 antibody [12G10] ab30394



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Overview

Product name Anti-Integrin beta 1 antibody [12G10]

Description Mouse monoclonal [12G10] to Integrin beta 1

Host species Mouse

Tested applications Suitable for: ICC/IF, Flow Cyt, WB

Species reactivity Reacts with: Human

Does not react with: Mouse, Rat

Immunogen Full length native protein (purified) corresponding to Integrin beta 1. Purified from HT1080

fibrosarcoma cell extract.

Epitope The 12G10 epitope has been identified to be within the von Willebrand factor type A domain of

the ß-subunit (PubMed ID: 15632175).

Positive control WB: HT1080 whole cell lysate. ICC/IF: HeLa cells. Flow Cyt: HeLa cells. ICC: HeLa cells

General notes This monoclonal antibody to integrin beta 1 has been knockout validated in ICC/IF and flow

cytometry. The expected signal was observed in wild type cells and was not seen in knockout

cells.

The 12G10 monoclonal has been shown to increase ligand binding and enhances $\alpha5\beta1$ integrinfibronectin interactions (PubMed ID: 7537221). The antibody promotes cell attachment and spreading when binding to $\alpha5\beta1$ integrin but inhibits these processes when binding to $\alpha4\beta1$

integrin (PubMed ID: 15632175).

This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or

conjugation for your experiments, please contact $\underline{\text{orders@abcam.com}}.$

The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets

your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be

found below, along with publications, customer reviews and Q&As

Properties

1

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long

term. Avoid freeze / thaw cycle.

Storage buffer pH: 7.40

Preservative: 0.02% Sodium azide

Constituent: PBS

Purity Protein G purified

Purification notes Purified from tissue culture supernatant.

Primary antibody notesThis antibody also enhances alpha 5 - beta 1 - fibronectin interactions.

Clonality Monoclonal
Clone number 12G10

Myeloma x63-Ag8.653

lsotype lgG1

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab30394 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ICC/IF	★★★★★ (3)	Use a concentration of 1 - 10 µg/ml.
Flow Cyt	★★★★ (2)	Use a concentration of 1 μ g/ml. <u>ab170190</u> - Mouse monoclonal lgG1, is suitable for use as an isotype control with this antibody.
WB	**** <u>(2)</u>	Use a concentration of 5 µg/ml. Detects a band of approximately 130 kDa (predicted molecular weight: 88 kDa).

Target

Function

Integrins alpha-1/beta-1, alpha-2/beta-1, alpha-10/beta-1 and alpha-11/beta-1 are receptors for collagen. Integrins alpha-1/beta-1 and alpha-2/beta-2 recognize the proline-hydroxylated sequence G-F-P-G-E-R in collagen. Integrins alpha-2/beta-1, alpha-3/beta-1, alpha-4/beta-1, alpha-4/beta-1, alpha-5/beta-1, alpha-8/beta-1, alpha-10/beta-1, alpha-11/beta-1 and alpha-V/beta-1 are receptors for fibronectin. Alpha-4/beta-1 recognizes one or more domains within the alternatively spliced CS-1 and CS-5 regions of fibronectin. Integrin alpha-5/beta-1 is a receptor for fibrinogen. Integrin alpha-1/beta-1, alpha-2/beta-1, alpha-6/beta-1 and alpha-7/beta-1 are receptors for lamimin. Integrin alpha-4/beta-1 is a receptor for VCAM1. It recognizes the sequence Q-I-D-S in VCAM1. Integrin alpha-9/beta-1 is a receptor for VCAM1, cytotactin and osteopontin. It recognizes the sequence A-E-I-D-G-I-E-L in cytotactin. Integrin alpha-3/beta-1 is a receptor for epiligrin, thrombospondin and CSPG4. Alpha-3/beta-1 may mediate with LGALS3 the stimulation by CSPG4 of endothelial cells migration. Integrin alpha-V/beta-1 is a receptor for vitronectin. Beta-1 integrins recognize the sequence R-G-D in a wide array of ligands. Isoform 2 interferes with isoform 1 resulting in a dominant negative effect on cell adhesion and migration (in vitro).

When associated with alpha-7/beta-1 integrin, regulates cell adhesion and laminin matrix deposition. Involved in promoting endothelial cell motility and angiogenesis. Involved in osteoblast compaction through the fibronectin fibrillogenesis cell-mediated matrix assembly process and the formation of mineralized bone nodules. May be involved in up-regulation of the activity of kinases such as PKC via binding to KRT1. Together with KRT1 and RACK1, serves as a platform for SRC activation or inactivation. Plays a mechanistic adhesive role during telophase, required for the successful completion of cytokinesis. Integrin alpha-3/beta-1 provides a docking site for FAP (seprase) at invadopodia plasma membranes in a collagen-dependent manner and hence may participate in the adhesion, formation of invadopodia and matrix degradation processes, promoting cell invasion. ITGA4:ITGB1 binds to fractalkine (CX3CL1) and may act as its coreceptor in CX3CR1-dependent fractalkine signaling (PubMed:23125415, PubMed:24789099). ITGA4:ITGB1 and ITGA5:ITGB1 bind to PLA2G2A via a site (site 2) which is

PubMed:24789099). ITGA4:ITGB1 and ITGA5:ITGB1 bind to PLA2G2A via a site (site 2) which is distinct from the classical ligand-binding site (site 1) and this induces integrin conformational changes and enhanced ligand binding to site 1 (PubMed:18635536, PubMed:25398877). ITGA5:ITGB1 acts as a receptor for fibrillin-1 (FBN1) and mediates R-G-D-dependent cell adhesion to FBN1 (PubMed:12807887, PubMed:17158881).

lsoform 5: lsoform 5 displaces isoform 1 in striated muscles.

(Microbial infection) Integrin ITGA2:ITGB1 acts as a receptor for human echoviruses 1 and 8 (PubMed:8411387). Acts as a receptor for cytomegalovirus/HHV-5 (PubMed:20660204). Acts as a receptor for Epstein-Barr virus/HHV-4 (PubMed:17945327). Integrin ITGA5:ITGB1 acts as a receptor for human parvovirus B19 (PubMed:12907437). Integrin ITGA2:ITGB1 acts as a receptor for human rotavirus (PubMed:12941907). Acts as a receptor for mammalian reovirus (PubMed:16501085). In case of HIV-1 infection, integrin ITGA5:ITGB1 binding to extracellular viral Tat protein seems to enhance angiogenesis in Kaposi's sarcoma lesions (PubMed:10397733).

Isoform 1 is widely expressed, other isoforms are generally coexpressed with a more restricted distribution. Isoform 2 is expressed in skin, liver, skeletal muscle, cardiac muscle, placenta, umbilical vein endothelial cells, neuroblastoma cells, lymphoma cells, hepatoma cells and astrocytoma cells. Isoform 3 and isoform 4 are expressed in muscle, kidney, liver, placenta, cervical epithelium, umbilical vein endothelial cells, fibroblast cells, embryonal kidney cells, platelets and several blood cell lines. Isoform 4, rather than isoform 3, is selectively expressed in peripheral T-cells. Isoform 3 is expressed in non-proliferating and differentiated prostate gland epithelial cells and in platelets, on the surface of erythroleukemia cells and in various hematopoietic cell lines. Isoform 5 is expressed specifically in striated muscle (skeletal and cardiac muscle).

Belongs to the integrin beta chain family.

Contains 1 VWFA domain.

The cysteine residues are involved in intrachain disulfide bonds.

Cell membrane, sarcolemma. Cell junction. In cardiac muscle, isoform 5 is found in costameres and intercalated disks and Cell membrane. Cell projection, invadopodium membrane. Cell projection, ruffle membrane. Recycling endosome. Melanosome. Cleavage furrow. Cell projection, lamellipodium. Cell projection, ruffle. Cell junction, focal adhesion. Cell surface. Isoform 2 does not localize to focal adhesions. Highly enriched in stage I melanosomes. Located on plasma membrane of neuroblastoma NMB7 cells. In a lung cancer cell line, in prometaphase and metaphase, localizes diffusely at the membrane and in a few intracellular vesicles. In early telophase, detected mainly on the matrix-facing side of the cells. By mid-telophase, concentrated to the ingressing cleavage furrow, mainly to the basal side of the furrow. In late telophase, concentrated to the extending protrusions formed at the opposite ends of the spreading daughter cells, in vesicles at the base of the lamellipodia formed by the separating daughter cells. Colocalizes with ITGB1BP1 and metastatic suppressor protein NME2 at the edge or peripheral

Tissue specificity

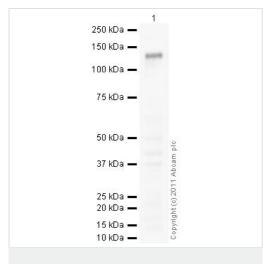
Sequence similarities

Post-translational modifications

Cellular localization

ruffles and lamellipodia during the early stages of cell spreading on fibronectin or collagen. Translocates from peripheral focal adhesions sites to fibrillar adhesions in a ITGB1BP1-dependent manner. Enriched preferentially at invadopodia, cell membrane protrusions that correspond to sites of cell invasion, in a collagen-dependent manner. Localized at plasma and ruffle membranes in a collagen-independent manner.

Images



Western blot - Anti-Integrin beta 1 antibody [12G10] (ab30394)

Anti-Integrin beta 1 antibody [12G10] (ab30394) at 5 μ g/ml + HT 1080 (Human fibrosarcoma) Whole Cell Lysate at 20 μ g

Secondary

Goat polyclonal Secondary Antibody to Mouse IgG - H&L (HRP), pre-adsorbed at 1/5000 dilution

Performed under reducing conditions.

Predicted band size: 88 kDa **Observed band size:** 130 kDa

Exposure time: 20 minutes

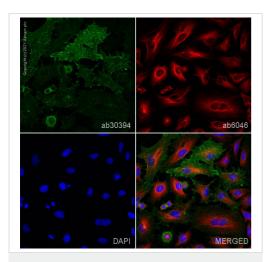
Integrin beta 1 contains a number of potential glycosylation sites (SwissProt) which may explain its migration at a higher molecular weight than predicted.

MERGED ab30394

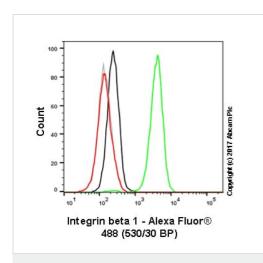
Capyright (© 2015 Abrain ple

Immunocytochemistry/ Immunofluorescence - Anti-Integrin beta 1 antibody [12G10] (ab30394) ab30394 staining Integrin beta 1 in wild-type HAP1 cells (top panel) and Integrin beta 1 knockout HAP1 cells (bottom panel).

The cells were fixed with 4% formaldehyde (10min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab30394 at 10 μ g/ml concentration and **ab202272** at 1/250 dilution (shown in pseudo colour red) overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to Mouse IgG (Alexa Fluor[®] 488) (**ab150117**) at 2 μ g/ml (shown in green). Nuclear DNA was labeled in blue with DAPI.



Immunocytochemistry/ Immunofluorescence - Anti-Integrin beta 1 antibody [12G10] (ab30394)



Flow Cytometry - Anti-Integrin beta 1 antibody [12G10] (ab30394)

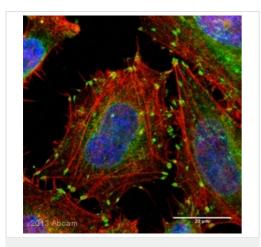
ab30394 staining Integrin beta 1 in HeLa cells. The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% PBS-Tween for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab30394 at 5µg/ml and ab6046, Rabbit polyclonal to beta Tubulin - Loading Control. Cells were then incubated with ab150117, Goat polyclonal Secondary Antibody to Mouse IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and ab150080, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 594) at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue).

Also suitable in cells fixed with 4% paraformaldehyde (10 min).

Image was acquired with a confocal microscope (Leica-Microsystems TCS SP8) and a single confocal section is shown.

Overlay histogram showing HAP1 wildtype (green line) and HAP1-ITGB1 knockout cells (red line) stained with ab30394.

Live HAP1 wildtype and HAP1-ITGB1 knockout cells were incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (ab30394, 1µg/0.5x10⁶ cells) for 30 min at 22°C. A mouse lgG1 isotype control antibody (ab170190) was used at the same concentration and conditions as the primary antibody (HAP1 wildtype - black line, HAP1-ITGB1 knockout - grey line). Unlabeled sample was also used as a control (this line is not shown for the purpose of simplicity). Acquisition of >5,000 events were collected using a 50 mW Blue laser (488nm) and 530/30 bandpass filter.

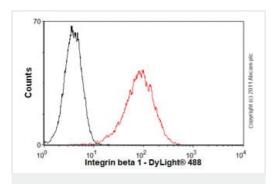


Immunocytochemistry/ Immunofluorescence - Anti-Integrin beta 1 antibody [12G10] (ab30394)

This image is courtesy of an anonymous abreview.

ab30394 at 1/500 staining Integrin beta 1 in HeLa (Human epithelial cell line from cervix adenocarcinoma) cells by Immunocytochemistry/ Immunofluorescence.

Cells were fixed in paraformaldehyde. The secondary used was an Alexa Fluor $^{\circledR}$ 488 Goat anti mouse (H + L), used at a 1/500 dilution. Actin filaments (Phalloidin-TRITC) and DNA (DAPI, blue) are also shown

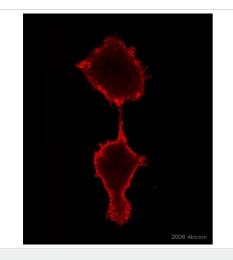


Flow Cytometry - Anti-Integrin beta 1 antibody [12G10] (ab30394)

Overlay histogram showing HeLa (Human epithelial cell line from cervix adenocarcinoma) cells stained with ab30394 (red line).

The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab30394, $1\mu g/1x10^6$ cells) for 30 min at 22°C. The secondary antibody used was a goat anti-mouse DyLight[®] 488 (lgG; H+L) <u>ab96879</u> at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse lgG1 [ICIGG1] (<u>ab91353</u>, $2\mu g/1x10^6$ cells) used under the same conditions. Acquisition of >5,000 events was performed.

This antibody gave a positive signal in HeLa cells fixed with 4% paraformaldehyde (10 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.



Immunocytochemistry/ Immunofluorescence - Anti-Integrin beta 1 antibody [12G10] (ab30394)

This image is courtesy of an anonymous abreview.

ab30394 at 10 μ g/ml staining Integrin beta 1 in HT1080 (Human fibrosarcoma cell line) cells by Immunocytochemistry/ Immunofluorescence.

Cells were fixed in paraformal dehyde. The secondary used was an Alexa Fluor $^{\! (\! R \!)}$ 555 Donkey anti mouse (H + L), used at a 1/500 dilution.

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