15-288-21523
Acris Antibodies GmbH
Schillerstr. 5
D-32052 Herford
Phone: +49-5221-34606-0
Fax: +49-5221-34606-11
info@acris-antibodies.com

Polyclonal Antibody to CDC14B1 - Aff - Purified

Catalog No.: 15-288-21523

Quantity: 0.1 mg

Background: Vinculin is an ubiquitously expressed cytoskeletal protein (~130 kDa) involved in cell adhesion and cell migration. The vinculin protein consists of a globular head domain connected to an elongated tail region by a proline-rich domain. The head region contains binding sites for two cytoskeletal proteins, α-actinin and talin, as well as a binding site for the tail region of vinculin itself. The tail region contains binding sites for actin, the cytoskeletal protein, paxillin, and PI(4,5)P2. In the inactive state the head region of vinculin is bound to the tail region, resulting in inaccessibility of the other protein binding sites. Binding of PI(4,5)P2 releases the head-tail interaction allowing binding of other proteins to vinculin. These regulatory events play an important role in the formation, maintenance, and breakdown of focal adhesions that occur during cell adhesion and migration. In addition to these protein binding sites, the head and tail regions of vinculin have multiple potential phosphorylation sites. Phosphorylation of vinculin on tyrosine residue 100 has been shown to play a role in cell spreading; however, it does not affect the interaction between vinculin and actin or cellular localization. The mechanism by which vinculin is phosphorylated on pY100 is unclear.

Host / Isotype: Chicken

Immunogen: Chemically synthesized phosphopeptide derived from a region of human vinculin that contains tyrosine 100
Remarks: The sequence is conserved in mouse and chicken.

Format: **State:** Liquid Ig fraction
Purification: Epitope-specific affinity chromatography. The antibody has been negatively preadsorbed using a non-phosphopeptide corresponding to the site of phosphorylation to remove antibody that is reactive with non-phosphorylated vinculin.
Buffer System: Dulbecco's phosphate buffered saline (without Mg2+ and Ca2+), pH 7.3 (+/- 0.1), 50% glycerol, with 1.0 mg/mL BSA (IgG, protease free) as a carrier, containing 0.05 % sodium azide as preservative

Applications: Western blot (1:1000).
Positive Control Used: COS cells co-transfected with activated Src and His-tagged chicken vinculin cDNA were treated with vanadate for 24 hr.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: This antibody detects Vinculin.
Species: Human, Mouse, Chicken.
Others not tested.

Storage: Store the antibody at -20 °C. Can be shipped at 2 - 8 °C.
Avoid repeated freezing and thawing.
Centrifuge vial before opening.
Shelf life: One year from despatch.

General References:
Zhang, Z., et al. (2004) The phosphorylation of vinculin on tyrosine residues 100 and 1065, mediated by SRC kinases, affects cell spreading. Mol. Biol. Cell. 15(9):4234-4247.
Subauste, M.C., et al. (2004) Vinculin modulation of paxillin-FAK interactions regulates ERK to control survival and motility. J. Cell. Biol. 165(3):371-381.
von Wichert, G., et al. (2003) Force-dependent integrin-cytoskeleton linkage formation requires downregulation of focal complex dynamics by Shp2. EMBO J. 22(19):5023-5035.
Zaidel-Bar, R., et al. (2003) Early molecular events in the assembly of matrix adhesions at the leading edge of migrating cells. J. Cell Sci. 116(Pt 22):4605-4613.
Ito, S., et al. (1983) Vinculin phosphorylation by the src kinase. Interaction of vinculin

Protocols:

Western Blotting Procedure

1. Lyse approximately 10e7 cells in 0.5 mL of ice cold Cell Lysis Buffer (formulation provided below). This buffer, a modified RIPA buffer, is suitable for recovery of most proteins, including membrane receptors, cytoskeletal-associated proteins, and soluble proteins. Other cell lysis buffer formulations, such as Laemmli sample buffer and Triton-X 100 buffer, are also compatible with this procedure. Additional optimization of the cell stimulation protocol and cell lysis procedure may be required for each specific application.
2. Remove the cellular debris by centrifuging the lysates at 14,000 x g for 10 minutes. Alternatively, lysates may be ultracentrifugedat 100,000 x g for 30 minutes for greater clarification.
3. Carefully decant the clarified cell lysates into clean tubes and determine the protein concentration using a suitable method, such as the Bradford assay. Polypropylene tubes are recommended for storing cell lysates.
4. React an aliquot of the lysate with an equal volume of 2x Laemmli Sample Buffer (125 mM Tris, pH 6.8, 10% glycerol, 10% SDS, 0.006% bromophenol blue, and 130 mM dithiothreitol [DTT]) and boil the mixture for 90 seconds at 100°C.
5. Load 10-30 µg of the cell lysate into the wells of an appropriate single percentage or gradient minigel and resolve the proteins by SDS-PAGE.
6. In preparation for the Western transfer, cut a piece of PVDF membrane slightly larger than the gel. Soak the membrane in methanol for 1 minute, then rinse with ddH2O for 5 minutes. Alternatively, nitrocellulose may be used.
7. Soak the membrane, 2 pieces of Whatman paper, and Western apparatus sponges in transfer buffer (formulation provided below) for 2 minutes.
8. Assemble the gel and membrane into the sandwich apparatus.
9. Transfer the proteins at 140 mA for 60-90 minutes at room temperature.
10. Following the transfer, rinse the membrane with Tris buffered saline for 2 minutes.
11. Block the membrane with blocking buffer (formulation provided below) for one hour at room temperature or overnight at 4°C.
12. Incubate the blocked blot with primary antibody at a 1:1000 dilution in Tris buffered saline supplemented with 3% BSA and 0.1% Tween 20 for one hour at room temperature or overnight at 4 °C.13. Wash the blot with several changes of Tris buffered saline supplemented with 0.1% Tween 20.
14. Detect the antibody band using an appropriate secondary antibody, such as goat F(ab)2 anti-rabbit IgG alkaline phosphatase conjugate or goat F(ab)2 anti-rabbit IgG horseradish peroxidase conjugate in conjunction with your chemiluminescence reagents and instrumentation.

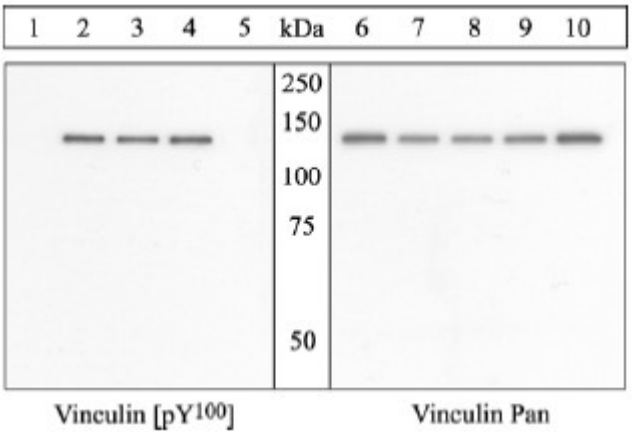
Cell Lysis Buffer Formulation:
10 mM Tris, pH 7.4
100 mM NaCl
1 mM EDTA
1 mM EGTA
1 mM NaF
20 mM Na4P2O7
2 mM Na3VO4
0.1% SDS
0.5% sodium deoxycholate
1% Triton-X 100
10% glycerol
1 mM PMSF (made from a 0.3 M stock in DMSO)
or 1 mM AEBSF (water soluble version of PMSF)
60 µg/mL aprotinin
10 µg/mL leupeptin
1 µg/mL pepstatin
(alternatively, protease inhibitor cocktail such as Sigma Cat. # P2714 may be used)

Transfer Buffer Formulation:
2.4 gm Tris base
14.2 gm glycine
200 mL methanol
Q.S. to 1 liter, then add 1 mL 10% SDS.
Cool to 4°C prior to use.

Tris Buffered Saline Formulation:
20 mM Tris-HCl, pH 7.4
0.9% NaCl

Blocking Buffer Formulation:
100 mL Tris buffered saline
5 gm BSA
0.1 mL Tween 20

Pictures:



Peptide Competition Lysates were prepared from COS cells co-transfected with activated Src and His-tagged chicken vinculin cDNA which were either untreated (1 and 6) or treated with vanadate for 24 hr (2-5 and 7-10). Following immunoprecipitation of vinculin with an anti-His monoclonal antibody, proteins were resolved by SDS-PAGE on an 8% polyacrylamide gel and transferred to PVDF. Membranes were blocked with a 5% BSA-TBST buffer for one hour at room temperature and incubated with vinculin [pY100] antibody for two hours at room temperature in 3% BSA-TBST buffer, following prior incubation with: no peptide (1, 2), the non-phosphopeptide corresponding to the immunogen (3), a generic phosphotyrosine-containing peptide (4), or, the phosphopeptide immunogen (5). After washing, membranes were incubated with goat F(ab')2 anti-rabbit IgG HRP conjugate in 3% BSA-TBST buffer, and bands were detected using the Pierce SuperSignal TM method. The blot was reprobed with an antibody against total vinculin to show equal loading (6-10). The data show that only the peptide corresponding to vinculin [pY100] blocks the antibody signal, thereby demonstrating the specificity of the antibody. In addition, no competition was observed following incubation with peptides corresponding to vinculin [pY882 or pY1065] (not shown).