Rabbit (polyclonal) Anti-Vinculin [pY^{822}] Phosphospecific Antibody, Unconjugated

PRODUCT ANALYSIS SHEET

Catalog Number:	44-1080G (10 mini-blot size)
Lot Number:	See product label
Volume:	100 µL
Form of Antibody:	Rabbit polyclonal immunoglobulin in Dulbecco's phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.3 (+/- 0.1), 50% glycerol, with 1.0 mg/mL BSA (IgG, protease free) as a carrier.
Preservative:	0.05% sodium azide (Caution: sodium azide is a poisonous and hazardous substance. Handle with care and dispose of properly.)
Purification:	Purified from rabbit serum by 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. The final product is generated by affinity chromatography using a vinculin-derived peptide that is phosphorylated at tyrosine 822.
Immunogen:	The antiserum was produced against a chemically synthesized phosphopeptide derived from a region of human vinculin that contains tyrosine 822. The sequence is conserved in mouse, rat, and chicken.
Target Summary:	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 linker region possesses binding sites for two adaptor proteins, vinexin and ponsin, that may connect growth factor receptors with integrin signaling pathways, and a binding site for an actin-bundling protein, VASP. The tail region contains binding sites for actin, the cytoskeletal protein, paxillin, and $PI(4,5)P_2$. 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 $PI(4,5)P_2$ 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. Data indicate the possible involvement of phosphorylation of tyrosine 822 of vinculin with the integrity/strength of integrin-mediated focal adhesions.
Reactivity:	Chicken vinculin. Human, mouse, and rat vinculin (100% homologous) have not been tested, but are expected to react.
Applications:	The antibody has been used for Western blotting applications.
Suggested Working Dilutions:	For Western blotting applications, we recommend using the antibody at a 1:1000 dilution. The optimal antibody concentration should be determined empirically for each specific application.
Storage:	Store at –20°C. We recommend a brief centrifugation before opening to settle vial contents. Then, apportion into working aliquots and store at –20°C. For shipment or short-term storage (up to one week), 2-8°C is sufficient.
Expiration Date:	Expires one year from date of receipt when stored as instructed.

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Positive Control Used: Chick Embryo Fibroblasts (CEFs) transfected with activated Src.

Related Products:

Antibodies:

Integrin α4 [pS^{988}], Cat. # 44-864
Integrin β1 [pS^{785}], Cat. # 44-870G
Integrin β1 [pTpT$^{788/789}$], Cat. # 44-872G
Integrin β3 [pY^{773}], Cat. # 44-876G
Integrin β3 [pY^{785}], Cat. # 44-878
FAK [pY^{397}], Cat. # 44-624G
FAK [pY^{576}], Cat. # 44-652G
FAK [pS^{843}], Cat. # 44-594G
Paxillin [pY^{31}], Cat. # 44-720G
Paxillin [pY^{118}], Cat. # 44-722G
PAK 1/2/3 [pS^{141}], Cat. # 44-940G
PAK 1/2/3 [pT^{423}], Cat. # 44-942G
Src [pY^{418}], Cat. # 44-660G
Src [pY^{529}], Cat. # 44-662G

Extracts: CEF cell extracts +/- Src, Cat. # 55-120

References:

Opaza Saez, A.M., et al. (2003) Tension development during contractile stimulation of smooth muscle requires recruitment of paxillin and vinculin to the membrane. Am. J. Physiol. Cell Physiol. (ePub ahead of print; October 22, 2003).

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.

Ziegler, W.H., et al. (2002) A lipid-regulated docking site on vinculin for protein kinase C. J. Biol. Chem. 277(9):7396-7404.

Zamir, E. and B. Geiger (2001) Molecular complexity and dynamics of cell-matrix adhesions. J. Cell Sci. 114(Pt 20):3583-3590.

Sechi, A.S. and J. Wehland (2000) The actin cytoskeleton and plasma membrane connection: PtdIns(4,5)P_2 influences cytoskeletal protein activity at the plasma membrane. J. Cell Sci. 113(Pt 21):3685-3695.

Goldmann, W.H., et al. (1998) Differences in elasticity of vinculin-deficient F9 cells measured by magnetometry and atomic force spectroscopy. Exp. Cell Res. 239(2):235-242.

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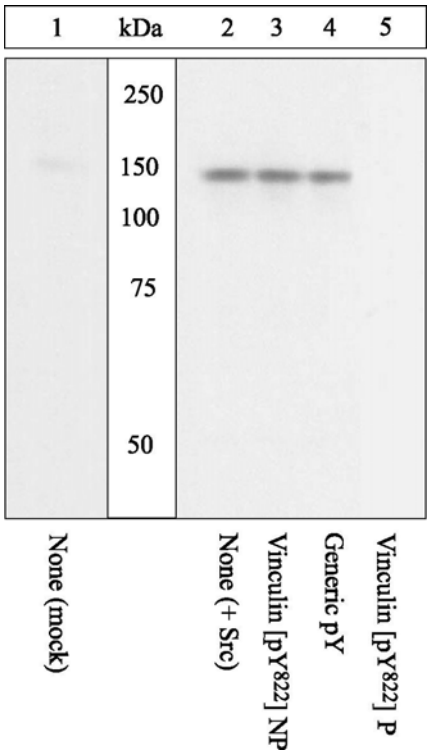

Peptide Competition

Lysates prepared from CEFs left untransfected (1) or transfected with Src (2-5) were resolved by SDS-PAGE on a 10% 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 [pY^{822}] antibody for one hour 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 (Cat. # ALI4404) in 3% BSA-TBST buffer, and bands were detected using the Pierce SuperSignal™ method.

The data show that only the peptide corresponding to vinculin [pY^{822}] blocks the antibody signal, thereby demonstrating the specificity of the antibody. The data also show that vinculin is highly phosphorylated in the presence of activated Src.

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Western Blotting Procedure

1. Lyse approximately 10^7 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. This cell lysis buffer formulation is available as a separate product which requires supplementation with protease inhibitors immediately prior to use (Cat. # FNN0011). 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 ultracentrifuged at 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 ddH_2O for 5 minutes. Alternatively, nitrocellulose may be used.
7. Soak the PVDF 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 (Cat. # ALI4405) or goat $F(ab')_2$ anti-rabbit IgG horseradish peroxidase conjugate (Cat. # ALI4404) 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 $Na_4P_2O_7$
2 mM Na_3VO_4
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

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