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ProductInformation

Anti-phospho-Vinculin [pTyr^{822}]
Developed in Rabbit, Affinity Isolated Antibody

Product Number **V 4889**

Product Description

Anti-phospho-Vinculin [pTyr^{822}] is developed in rabbit using a synthetic phosphorylated peptide derived from the region of vinculin that contains tyrosine 822 as immunogen. The antiserum is affinity purified using epitope-specific affinity chromatography. The antibody is preadsorbed to remove any reactivity toward a non-phosphorylated vinculin.

The antibody detects chicken vinculin. Human, mouse, and rat vinculin (100% homologous) have not been tested, but are expected to react. It has been used in immunoblotting applications.

Vinculin is a 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)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.

Data indicate the possible involvement of phosphorylation of tyrosine 822 of vinculin with the integrity/strength of integrin mediated focal adhesions.

Reagent

The antibody is supplied as a solution in Dulbecco's phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.3, with 50% glycerol, 1.0 mg/ml BSA (IgG and protease free) and 0.05% sodium azide.

Precautions and Disclaimer

Due to the sodium azide content, a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.

Storage/Stability

Store at –20 °C. Due to the presence of 50% glycerol the antibody will remain in solution. For extended storage, centrifuge the vial briefly before opening and prepare working aliquots. The antibody is stable for at least six months when stored appropriately. Working dilutions should be discarded if not used within 12 hours.

Product Profile

The supplied reagent is sufficient for 10 blots.

A recommended working dilution of 1:1000 is determined by immunoblotting using chick embryo fibroblasts (CEFs) transfected with activated Src.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working concentration by titration test.

Results

Peptide Competition

1. Lysates prepared from CEFs left untransfected (Lane 1) or transfected with Src (Lanes 2-5) were resolved by SDS-PAGE on a 10% polyacrylamide gel and transferred to PVDF.
2. Membranes were blocked with a 5% BSA-TBST buffer overnight at 4 °C.

3. After blocking, membranes were preincubated with different peptides as follow:
   Lane 1&2 no peptide
   Lane 3 non-phosphorylated peptide corresponding to the immunogen
   Lane 4 a generic phosphotyrosine containing peptide
   Lane 5 immunogen
4. After preincubation membranes were incubated with 0.50 µg/mL vinculin [pTyr^{822}] antibody for one hour at room temperature in a 3% BSA-TBST buffer.
5. After washing, membranes were incubated with goat F(ab')$_2$ anti-rabbit IgG alkaline phosphatase and signals were detected.

The data in Figure 1 show that only the peptide corresponding to vinculin [pTyr^{822}] blocks the antibody signal, demonstrating the specificity of the antibody.

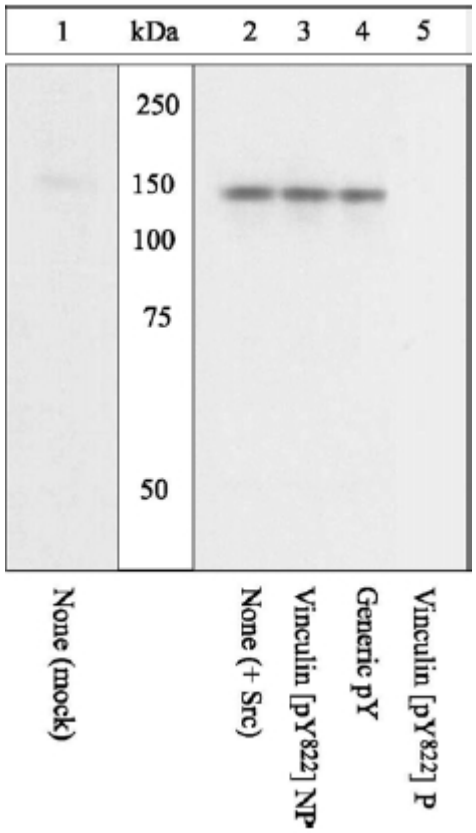

Figure 1 Peptide Competition

References

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