

Rabbit (polyclonal) Anti-Paxillin [pY31] Phosphospecific Antibody, Unconjugated

Store at 2°C to 8°C (short-term), or -20°C (long-term)

Catalog Number: 44-720G

Pub. No. MAN0010094 Rev. 1.00

Clonality: Polyclonal

Quantity: 10 mini-blot size

Volume: 100 µL

Host/Class: Rabbit IgG

Reactivity: Human Paxillin [pY31]

Predicted Reactivity: Human, Mouse, Chicken

Product description

Paxillin is a 68 kDa cytoskeletal adapter protein involved in organization and function of focal adhesions, which are critical to cell adhesion and migration. This in turn plays a role in a wide variety of processes including embryogenesis, organogenesis, wound repair, inflammation and cancer. Paxillin contains LD motifs, LIM domains, SH3 and SH2 binding domains that serve as docking sites for cytoskeletal proteins, tyrosine kinases (e.g., FAK, Pyk2, Src), serine/threonine kinases, GTPase activating proteins and other adaptor proteins (e.g., actin, vinculin, Crk). Tyrosine 31, a Crk binding site of paxillin, is phosphorylated during integrin-mediated cell adhesion.

Product specifications

Immunogen: A chemically synthesized phosphopeptide derived from a region of human paxillin containing tyrosine 31.

Purification: Antibody negatively preadsorbed using a non-phosphopeptide then purified by epitope-specific affinity chromatography

Lot: See product label

Product applications

The antibody has been used in western blotting applications (1:1000 dilution), and immunohistochemistry.^{1,4,7,8} Other applications may work but have not been tested.

Because conditions may vary, it is recommended that each investigator determine the optimal amount of antibody to be used for each application.

Storage and handling

Store the antibody at 2°C to 8°C for up to 1 week, or apportion into working aliquots and keep at -20°C for long-term storage. Avoid repeated freezing and thawing.

Stability

When stored as instructed, expires one year from date of receipt unless otherwise indicated on the Certificate of Analysis.

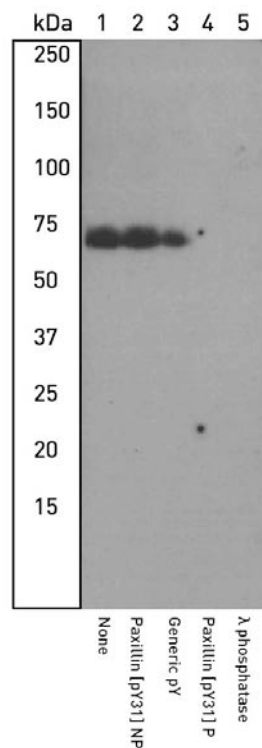


Figure 1 Peptide Competition.

A431 cells were treated with PMA (100 ng/mL for 30 minutes), and cell extracts were resolved on a 4-12% BisTris gel and transferred to PVDF. The membrane was blocked with 5% BSA-TBST for 1 hour at room temperature then incubated with the Paxillin [pY31] antibody overnight at 4°C in 3% BSA-TBST, following prior incubation with: no peptide (lanes 1 and 5), a non-phosphorylated peptide corresponding to the immunogen (lane 2), a generic phosphotyrosine-containing peptide (lane 3), or the phosphopeptide immunogen (lane 4). The blots were developed using chemiluminescence (ECL) method with a goat anti-rabbit IgG HRP conjugate (Cat. no. 656120).

Only the phosphopeptide corresponding to Paxillin [pY31] (lane 4) or pre-treatment with lambda phosphatase (lane 5) blocks the antibody signal, demonstrating the specificity of the antibody.

Positive controls used

A431 cells were treated with PMA.

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Storage buffer

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, and 0.05% sodium azide.



CAUTION! Sodium azide is extremely toxic and may react with lead and copper plumbing to form highly explosive metal azides. Properly dispose of solutions containing sodium azide. Read the Safety Data Sheet (SDS) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. SDSs are available at www.lifetechnologies.com/support.

References

1. Grace, E.A. and J. Busciglio (2003) Aberrant activation of focal adhesion proteins mediates fibrillar amyloid beta-induced neuronal dystrophy. *J. Neurosci.* 23(2):493-502).
2. Levkau, B., et al. (2002) Activation of metalloproteinases and their association with integrins: an auxiliary apoptotic pathway in human endothelial cells. *Cell Death Differ.* 9(12):1360-1367.
3. Manabe, Ri R., et al. (2002) GIT1 functions in a motile, multi-molecular signaling complex that regulates protrusive activity and cell migration. *J. Cell Sci.* 115(Pt 7):1497-1510.
4. Melendez, J., et al. (2002) Activation of Pyk2/Related Focal Adhesion Tyrosine Kinase and Focal Adhesion Kinase in cardiac remodeling. *J. Biol. Chem.* 277(47):45203-45210.
5. Ushio-Fukai, M., et al. (2001) Cholesterol detection inhibits epidermal growth factor receptor transactivation by angiotensin II in vascular smooth muscle cells - Role of cholesterol-rich microdomains and focal adhesions in angiotensin II signaling. *J. Biol. Chem.* 276(51):48269-48275.
6. Weinberg, J.M., et al. (2001) Energetic determinants of tyrosine phosphorylation of focal adhesion proteins during hypoxia/reoxygenation of kidney proximal tubules. *Am. J. Pathol.* 158(6):2153-2164.
7. Sussman, M.A., et al. (2000) Altered focal adhesion regulation correlates with cardiomyopathy in mice expressing constitutively active rac1. *J. Clin. Invest.* 105(7):875-886.
8. Nakamura, K., et al. (2000) Tyrosine phosphorylation of paxillin α is involved in temporospatial regulation of paxillin-containing focal adhesion formation and F-actin organization in motile cells. *J. Biol. Chem.* 275(35):27155-27164.

References, continued

9. Schaller, M.D. and J.T. Parsons (1995) pp125FAK-dependent tyrosine phosphorylation of paxillin creates a high-affinity binding site for Crk. *Mol. Cell. Biol.* 15(5):2635-2645.

Related products

Product Name	Quantity	Cat. No.
Paxillin [pY118] Polyclonal Antibody, Rabbit	10 blots	44-722G
FAK PSSA Antibody Sample Pack	5 vials	44-631G
Pyk2 Antibody Sampler Pack	4 vials	44-638G

Product documentation

To obtain a Certificate of Analysis or Safety Data Sheets (SDSs), visit www.lifetechnologies.com/support.

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Explanation of symbols

Symbol	Description	Symbol	Description	Symbol	Description
	Manufacturer		Catalog number		Batch code
	Use by		Temperature limitation		
	Consult instructions for use		Caution, consult accompanying documents		

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