Rabbit (polyclonal) Anti-Paxillin [pS^{126}] Phosphospecific Antibody, Unconjugated

PRODUCT ANALYSIS SHEET

Catalog Number:	441022G (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 sequential epitope-specific 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 paxillin. The final product is generated by affinity chromatography using a paxillin-derived peptide that is phosphorylated at serine 126.
Immunogen:	The antiserum was produced against a chemically synthesized phosphopeptide derived from the region of human paxillin that contains serine 126. The sequence is conserved in mouse and chicken.
Target Summary:	Paxillin is a 68 kDa cytoskeletal adapter protein involved in organization and function of focal adhesions, which are critical to cell adhesion and migration. These cellular events in turn play 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, serine kinases (e.g., FAK, Pyk2, Src), serine/threonine kinases, GTPase activating proteins and other adaptor proteins (e.g., actin, vinculin, Crk). Serine 126 of paxillin is phosphorylated as a result of Raf stimulation, through the Raf → MEK → ERK pathway.
Reactivity:	Mouse and human (100% homologous) paxillin. Chicken (100% homologous) has not been tested, but is expected to react.
Applications:	The antibody has been used in Western blotting. Other applications may work but have not been tested.
Suggested Working Dilutions:	For Western blotting applications, we recommend using the antibody at a 1:1000 starting dilution. The exact concentration is not determined for each lot; however, the typical range is 0.1-1.0 mg/mL. 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.
Positive Controls Used:	RAW 264.7 cells treated with LPS or 293 cells transfected with wild-type EGFP-tagged human paxillin.

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This antibody is manufactured under a licensed process covered by Patent # 5, 599, 681.

PI441022G (Rev 11/08) DCC-08-1089

Related Products:

Antibodies:

Paxillin [pY^{31}], Cat. # 44720G

Paxillin [pY^{118}], Cat. # 44722G

Paxillin [pS^{178}], Cat. # 441026G

Sample Packs:

Pro-Growth Sample Pack, Cat. # 44587G

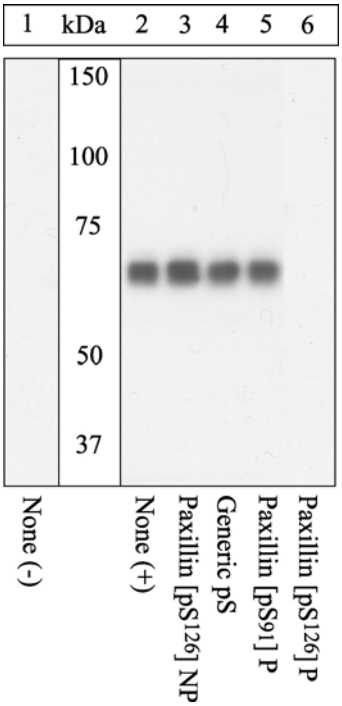
FAK pY Sample Pack, Cat. # 44631G

Pyk2 Sample Pack, Cat. # 44638G

References:

Woodrow, M.A., et al. (2003) Ras-induced serine phosphorylation of the focal adhesion protein paxillin is mediated by the Raf → MEK → ERK pathway. Exp. Cell. Res. 287(2):325-338.

Schaller, M.D. (2001) Paxillin: a focal adhesion-associated adaptor protein. Oncogene 20(44):6459-6472. Review.



Upregulation and Antibody-Peptide Competition

Extracts of RAW 264.7 cells unstimulated (1) or stimulated with 1 µg/mL LPS for 60 minutes (2-6) were resolved by SDS-PAGE on a 10% Tris-glycine gel and transferred to PVDF. The membrane was blocked with a 5% milk-TBST buffer for one hour at room temperature, and then incubated with the Paxillin [pS^{126}] antibody for two hours at room temperature in a 1% milk-TBST buffer, following its prior incubation with: no peptide (1, 2), the non-phosphorylated peptide corresponding to the phosphopeptide immunogen (3), a generic phosphoserine-containing peptide (4), a phosphopeptide corresponding to Paxillin [pS^{91}] (5), or the phosphopeptide immunogen (6). After washing, membranes were incubated with goat F(ab')$_2$ anti-rabbit IgG HRP conjugate (Cat. # ALI4404) and signals were detected using the Pierce SuperSignal™ method.

The data show that only the phosphopeptide corresponding to Paxillin [pS^{126}] blocks the antibody signal, demonstrating the specificity of the antibody. The data also show the upregulation of Paxillin [pS^{91}] phosphorylation upon stimulation with LPS in this cell system. No competition was seen following incubation with paxillin phosphopeptides to S^{130}, S^{178}, S^{380}, S^{455}, or S^{479} (not shown). The antibody was also shown to be specific using 293 cells transfected with wild-type EGFP-tagged human paxillin treated with EGF (not shown).

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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 (Invitrogen 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 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) overnight at 4°C or for one hour at room temperature..
12. Incubate the blocked blot with primary antibody at a 1:1000 starting dilution in Tris buffered saline supplemented with 1% non-fat dried milk and 0.1% Tween 20 overnight at 4°C or for two hours at room temperature.
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 non-fat dried milk
0.1 mL Tween 20

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