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Polyclonal Antibody to PED/PEA-15 pSer116 - Aff - Purified

Alternate names: Astrocytic Phosphoprotein PEA-15, PEA15, PED, Phosphoprotein Enriched in Diabetes

Catalog No.: BP7157

Quantity: 0.1 ml

Background: PED/PEA-15 (Phosphoprotein Enriched in Diabetes/Phosphoprotein Enriched in Astrocytes - 15 kDa) is a widely expressed 15 kDa protein comprised of an N-terminal region containing a canonical Death Effector Domain (DED) sequence and a nuclear export signal, and a C-terminal region containing two serine phosphorylation sites. PED/PEA-15 has been implicated in the regulation of multiple cellular processes including apoptosis, integrin activation, and insulin-sensitive glucose transport in insulin-responsive cells. Consistent with this latter function, expression of PED/PEA-15 is increased 2-3-fold in skeletal muscle and adipose cells of patients with Type II diabetes. PED/PEA-15 function is mediated through binding to multiple proteins, including ERK1&2, RSK2, Akt, FADD, and Caspase-8, which, in part, modulates their subcellular localization and access to potential substrates. A major function of PED/PEA-15 may be, therefore, to coordinate inputs from multiple pathways to achieve a unified cellular response. Phosphorylation of both serine 104 (a Protein Kinase C site) and serine 116 (a substrate of CaMKII and Akt) is required for PED/PEA-15 function.

Host: Rabbit

Immunogen: Chemically synthesized phosphopeptide derived from a region of human PED/PEA-15 that contains serine 116.
Remarks: The sequence is conserved in mouse and rat.

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 PED/PEA-15. The final product is generated by affinity chromatography using a PED/PEA-15-derived peptide that is phosphorylated at serine 116.
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 Controls Used: Cos7 cells transfected with PED/PEA-15 cDNA and stimulated with 100 ng/mL PMA; SK-BR-3 cells.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

For research and in vitro use only. Not for diagnostic or therapeutic work.
Material Safety Datasheets are available at www.acris-antibodies.com or on request.
Acris Antibodies is now part of the OriGene family. Learn more at www.origene.com
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Specificity: This antibody is specific for PED/PEA-15 pSer116.
Species: Human, Mouse, Rat.
Other species not tested.

Storage: Store at 2 - 8 °C up to one week or (in aliquots) at -20 °C for longer. Avoid repeated freezing and thawing.
Centrifuge vial before opening.
Shelf life: one year from despatch.

General Readings:

Renault, F., et al. (2003) The multifunctional protein PEA-15 is involved in the control of apoptosis and cell cycle in astrocytes. Biochem. Pharmacol. 66(8):1581-1588.
Chou, F.L., et al. (2003) PEA-15 binding to ERK1/2 MAP Kinases is required for its modulation of integrin activation. J. Biol. Chem. (in press) ePub Manuscript #M309322200.
Trencia, A., et al. (2003) Protein kinase B/Akt binds and phosphorylates PED/PEA-15, stabilizing its antiapoptotic action. Mol. Cell. Biol. 23(13):4511-4521.
Vaidyanathan, H. and J.W. Ramos (2003) RSK2 activity is regulated by its interaction with PEA-15. J. Biol. Chem. 278(34):32367-32372.
Condorelli, G., et al. (2002) Multiple members of the mitogen-activated protein kinase family are necessary for PED/PEA-15 antiapoptotic function. J. Biol. Chem. 277(13):11013-11018.
Xiao, C., et al. (2002) Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand-induced death-inducing signaling complex and its modulation by c-FLIP and PED/PEA-15 in glioma cells. J. Biol. Chem. 277(28):25020-25025.
Formstecher, E., et al. (2001) PEA-15 mediates cytoplasmic sequestration of ERK MAP Kinase. Dev. Cell 1(2):239-250.
Ramos, J.W., et al. (2000) Death effector domain protein PEA-15 potentiates Ras activation of Extracellular Signal Receptor-Activated Kinase by an adhesion-independent mechanism. Mol. Biol. Cell 11(9):2863-2872.

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 $Na_4P_2O_7$
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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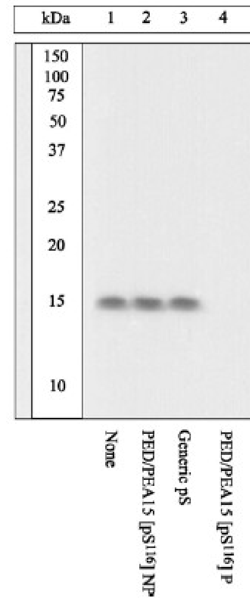
Material Safety Datasheets are available at www.acris-antibodies.com or on request.
Acris Antibodies is now part of the OriGene family. Learn more at www.origene.com

Pictures:

Peptide Competition Lysates prepared from SK-BR-3 cells were resolved by SDS-PAGE on an 18% polyacrylamide gel and transferred to PVDF. Membranes were blocked with a 5% BSA-TBST buffer for one hour at room temperature and incubated with PED/PEA-15 [pS116] antibody for one hour at room temperature in 3% BSA-TBST buffer, following prior incubation with: no peptide (1), the non-phosphopeptide corresponding to the immunogen (2), a generic phosphoserine-containing peptide (3), or, the phosphopeptide immunogen (4). 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 SuperSignalTM method.



Mutant experiments Lysates were prepared from PMA-stimulated Cos7 cells transfected with HA-tagged wild-type PED/PEA-15 (A), His-tagged S104A mutant PED/PEA-15 (B), and His-tagged S116A mutant PED/PEA-15 (C). Proteins were resolved by SDS-PAGE, transfered to PVDF, and blotted with PED/PEA-15 [pS116] antibody as above.