
Certificate of Analysis

Anti-phospho-PED/PEA-15 (Ser116)

(Affinity purified Rabbit polyclonal IgG)

Catalog # 07-865

Lot # 0604027255

Immunogen: Peptide corresponding to amino acid region encompassing the human, mouse, rat and frog phospho-PED/PEA-15 (Ser116).

Specificity: Recognizes phospho-PED/PEA-15 (Ser116), Mr. 15.

Species Cross-reactivity: Human and Mouse tested. Predicted to cross-react with Rat (100% homologous) based on sequence homology.

Formulation: 100 μ L of affinity purified rabbit polyclonal IgG in 50% storage buffer (PBS (without Mg^{2+} and Ca^{2+}), pH 7.3 containing 1.0 mg/mL BSA (IgG, protease free) and 0.05% sodium azide) and 50% glycerol.

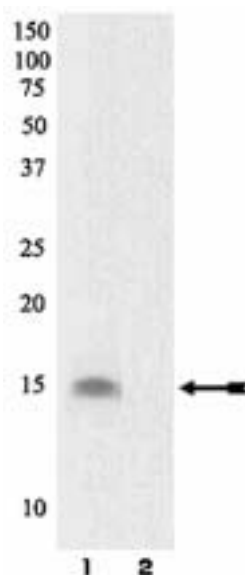
Storage and Stability: Stable for 2 years at $-20^{\circ}C$ from date of shipment.

Handling Recommendations: Upon first thaw, and prior to removing the cap, centrifuge the vial and gently mix the solution. Aliquot into microcentrifuge tubes and store at $-20^{\circ}C$. **Avoid repeated freeze/thaw cycles, which may damage IgG and affect product performance.** Note: Variability in freezer temperatures below $-20^{\circ}C$ may cause glycerol-containing solutions to become frozen during storage

**FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS**

Quality Control Testing

Immunoblot Analysis: A 1:1,000 dilution of this lot detected phospho-PED/PEA-15 in RIPA lysates from SK-BR-3 cell lysate.



Immunoblot Analysis

Lysates from untreated SK-BR-3 cells were resolved by electrophoresis, transferred to nitrocellulose, and probed with anti-phospho-PED/PEA-15 (Ser116). Lane 2 was pre-incubated with the blocking peptide immunogen corresponding to phospho-PED/PEA-15 (Ser116). Proteins were visualized via HRP and chemiluminescent detection. Arrow indicates phospho-PED/PEA-15.

General References:

1. Trecia A, et al., *Mol Cell Biol*, **23**: 4511-21, 2003.

Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on untreated SK-BR-3 lysate sample (cell lysis buffer: 50mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EDTA; 1mM PMSF; 1µg/ml each aprotinin, leupeptin, pepstatin; 1mM Na₃VO₄, 1mM NaF) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared 3% nonfat dry milk (Catalog # 20-200) in PBS with 0.05% Tween[®]-20 (PBS-MLK) for 20 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose with 1:1000 dilution of **anti-PED/PEA-15 (Ser116)**, diluted in freshly prepared PBS-MLK overnight with agitation at 4°C.
4. Wash the nitrocellulose twice with water.
5. Incubate the nitrocellulose in the secondary reagent of choice (a goat anti-rabbit HRP conjugated IgG, Catalog # 12-348, 1:5000 dilution was used) in PBS-MLK for 1.5 hours with agitation at room temperature.
6. Wash the nitrocellulose twice with water.
7. Wash the nitrocellulose in PBS-0.05% Tween[®]-20 for 3-5 minutes.
8. Rinse the nitrocellulose in 4-5 changes of water.
9. Use detection method of choice (enhanced chemiluminescence was used).