Anti-Phospho-Ezrin (T567)/Radixin (T564)/Moesin (T558) Antibody [PSH05-73] - BSA and Azide free

HA751023

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IHC-P
Molecular Wt:	Predicted band size: 69 kDa
Clone number:	PSH05-73

Description: The ezrin, radixin, and moesin (ERM) proteins function as linkers between the plasma membrane and the actin cytoskeleton and are involved in cell adhesion, membrane ruffling, and microvilli formation . ERM proteins undergo intra or intermolecular interaction between their amino- and carboxy-terminal domains, existing as inactive cytosolic monomers or dimers. Phosphorylation at a carboxy-terminal threonine residue (Thr567 of ezrin, Thr564 of radixin, Thr558 of moesin) disrupts the amino- and carboxy-terminal association and may play a key role in regulating ERM protein conformation and function. Phosphorylation at Thr567 of ezrin is required for cytoskeletal rearrangements and oncogene-induced transformation. Ezrin is also phosphorylated at tyrosine residues upon growth factor stimulation. Phosphorylation of Tyr353 of ezrin transmits a survival signal during epithelial differentiation.

Immunogen: Synthetic phosphopeptide corresponding to residues surrounding Thr567 of human ezrin protein.

Positive control: HeLa starved for 3 hours then add 100nM Calyculin A for 30 minutes cell lysate, NIH/3T3 starved for 24 hours then add 100nM Calyculin A for 30 minutes cell lysate, C6 treated with 100ng/mL Calyculin A for 1 hours cell lysate, mouse liver tissue, rat liver tissue.

Subcellular location: Cell membrane. Cell projection. Cytoplasm. Cytoskeleton. Membrane

Database links: SwissProt: P15311 Human | P26038 Human | P35241 Human | P26040 Mouse | P26041 Mouse | P26043 Mouse | P31977 Rat | O35763 Rat

Recommended Dilutions:

WB	1:2,000
IF-Cell	1:100
IHC-P	1:1,000

Storage Buffer: 1*PBS (pH7.4).

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

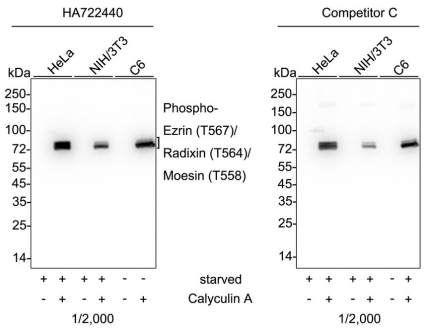

Fig1: Western blot analysis of Phospho-Ezrin (T567)/Radixin (T564)/Moesin (T558) on different lysates with Rabbit anti-Phospho-Ezrin (T567)/Radixin (T564)/Moesin (T558) antibody (HA751023) at 1/2,000 dilution and competitor's antibody at 1/2,000 dilution.

Lane 1: HeLa starved for 3 hours cell lysate
Lane 2: HeLa starved for 3 hours then add 100nM Calyculin A for 30 minutes cell lysate
Lane 3: NIH/3T3 starved for 24 hours cell lysate
Lane 4: NIH/3T3 starved for 24 hours then add 100nM Calyculin A for 30 minutes cell lysate
Lane 5: C6 cell lysate
Lane 6: C6 treated with 100ng/mL Calyculin A for 1 hours cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 69 kDa
Observed band size: 75/80 kDa

Exposure time: 9 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA751023) at 1/2,000 dilution and competitor's antibody at 1/2,000 dilution were used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

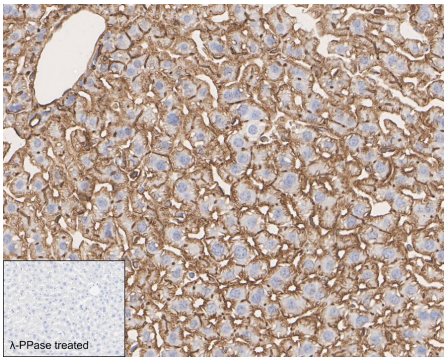

Fig2: Immunohistochemical analysis of paraffin-embedded mouse liver tissue untreated / treated with λpp with Rabbit anti-Phospho-Ezrin (T567)/Radixin (T564)/Moesin (T558) antibody (HA751023) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA751023) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

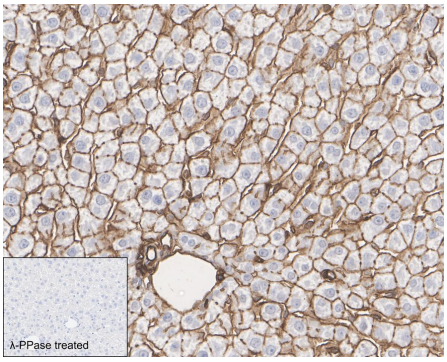

Fig3: Immunohistochemical analysis of paraffin-embedded rat liver tissue untreated / treated with λpp with Rabbit anti-Phospho-Ezrin (T567)/Radixin (T564)/Moesin (T558) antibody (HA751023) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA751023) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

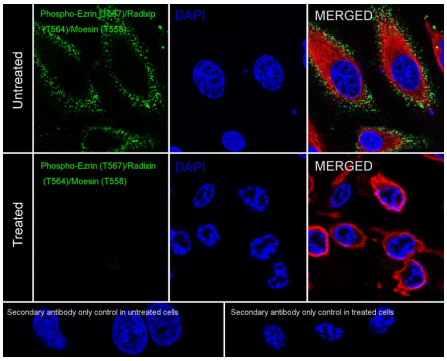

Fig4: Immunocytochemistry analysis of HeLa cells untreated (upper, positive) and HeLa cells treated with 1μM staurosporine for 3 hours (lower, negative) labeling Phospho-Ezrin (T567)/Radixin (T564)/Moesin (T558) with Rabbit anti-Phospho-Ezrin (T567)/Radixin (T564)/Moesin (T558) antibody (HA751023) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Phospho-Ezrin (T567)/Radixin (T564)/Moesin (T558) antibody (HA751023) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

Background References

1. Michie KA et al. Two Sides of the Coin: Ezrin/Radixin/Moesin and Merlin Control Membrane Structure and Contact Inhibition. Int J Mol Sci. 2019 Apr
2. Neisch AL et al. Ezrin, Radixin and Moesin: key regulators of membrane-cortex interactions and signaling. Curr Opin Cell Biol. 2011 Aug