Anti-Phospho-Raf1 (S621) Antibody [JJ085-05] ET1701-3

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Rat, Mouse
Applications:	WB, IHC-P
Molecular Wt:	Predicted band size: 73 kDa
Clone number:	JJ085-05

Description: Raf-1 is a ubiquitously expressed cytoplasmic protein with intrinsic serine/threonine kinase activity. Raf-1, or c-Raf, is the cellular homolog of v-Raf, the product of the transforming gene of the 3611 strain of murine sarcoma virus. The unregulated kinase activity of the v-Raf protein is associated with cellular transformation and mitogenesis. Raf-1 is normally suppressed by its regulatory N-terminal domain. Raf-1 is activated in response to a variety of tyrosine kinase receptors as well as in response to pp60v-Src expression. Specifically, Raf-1 is phosphorylated in the catalytic domain at Ser 338 and, to a lesser extent, Ser 339. This phosphorylation requires the co-activation of PI 3-kinase and the Ras signaling pathway. Raf-1 is also phosphorylated on Tyr 340 and 341, which induces the phosphorylation of MEK. Phosphorylation of Ser 621 is essential for the catalytic activity of Raf-1 and downregulation by c-AMP-dependent protein kinase A (PKA). PKA also phosphorylates Raf-1 on Ser 43 and Ser 259. PKA phosphorylation of Ser 259 inhibits Raf-1 and decreases the phosphorylation necessary for Raf-1 activation at Ser 338.

Immunogen: Synthetic phospho-peptide corresponding to residues surrounding Ser621 of Human Raf1 aa 591-640 / 648.

Positive control: Human skeletal muscle tissue lysates, rat trachea tissue, human muscle tissue, human kidney tissue.

Subcellular location: Cytoplasm, Cell membrane, Mitochondrion, Nucleus.

Database links: SwissProt: P04049 Human | Q99N57 Mouse | P11345 Rat

Recommended Dilutions:

WB	1:1,000-1:2,000
IHC-P	1:100-1:500

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

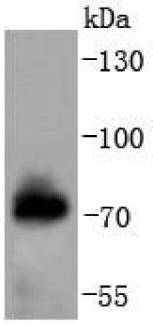

Fig1: Western blot analysis of Phospho-Raf1 (S621) on human skeletal muscle tissue lysates. Proteins were transferred to a PVDF membrane and blocked with 5% BSA in PBS for 1 hour at room temperature. The primary antibody (ET1701-3, 1/500) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:200,000 dilution was used for 1 hour at room temperature.

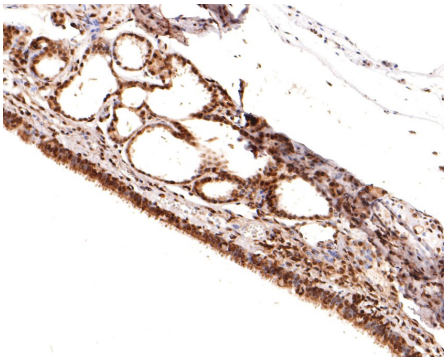

Fig2: Immunohistochemical analysis of paraffin-embedded rat trachea tissue using anti-Phospho-Raf1 (S621) antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1701-3, 1/400) for 30 minutes 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.

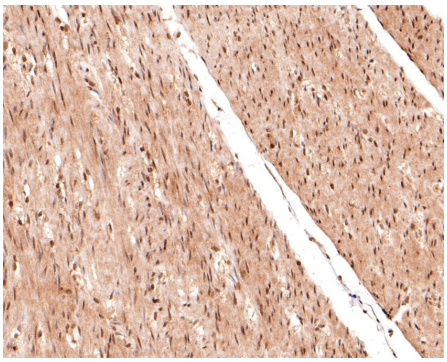

Fig3: Immunohistochemical analysis of paraffin-embedded human muscle tissue using anti-Phospho-Raf1 (S621) antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1701-3, 1/400) for 30 minutes 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.

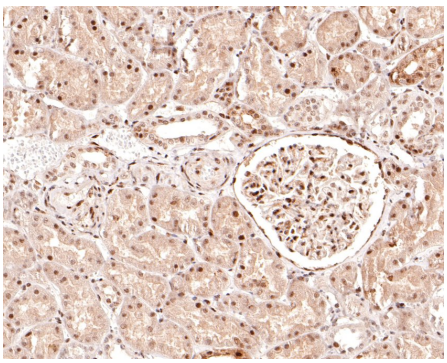

Fig4: Immunohistochemical analysis of paraffin-embedded human kidney tissue using anti-Phospho-Raf1 (S621) antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1701-3, 1/100) for 30 minutes 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.

Background References

1. K hler, M. et al. 2016. Activation loop phosphorylation regulates B-Raf in vivo and transformation by B-Raf mutants. The EMBO journal. 35: 143-61.
2. El-Chaar, NN. et al. 2014. Genomic classification of the RAS network identifies a personalized treatment strategy for lung cancer. Molecular oncology. 8: 1339-54.