bs-3280R **[Primary Antibody]**

Phospho-MLK3 (Thr277 + Ser281) Rabbit pAb

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DATASHEET

Host: Rabbit **Isotype:** IgG

Clonality: Polyclonal

GeneID: 4296 **SWISS:** Q16584

Target: Phospho-MLK3 (Thr277 + Ser281)

Immunogen: KLH conjugated synthesised phosphopeptide derived from human MLK3 around the phosphorylation site of Thr277/Ser281: K(p-T)TQM(p-S)AA.

Purification: affinity purified by Protein A

Concentration: 1mg/ml

Storage: 0.01M TBS (pH7.4) with 1% BSA, 0.02% Proclin300 and 50% Glycerol.
Shipped at 4°C. Store at -20°C for one year. Avoid repeated freeze/thaw cycles.

Background: Members of the mixed-lineage kinase (MLK) family (including MLK1, MLK2, MLK3, and dual leucine zipper kinase [DLK]) are serine/threonine protein kinases that are expressed in multiple cell types. MLK3 is activated by phosphorylation in response to stress stimuli (e.g., inflammatory responses, UV, chemical stress) that are coupled to the small GTPase, Cdc42/rac. MLK3 is a multifunctional kinase that plays an essential role in several signaling pathways, including mitogen-activated protein kinase (i.e. activation of JNK and p38), IkappaB/NFkappaB, and p70 S6 kinase. Indeed MLK3 signaling occurs through multiple signaling domains in this protein kinase including (from N- to C-terminal) a glycine-rich domain, Src homology 3 (SH3) domain, a kinase domain, a zipper domain, a Cdc42/rac interactive binding (CRIB) domain and a Pro/Ser/Thr-rich domain. Phosphorylation of MLK3 occurs on multiple residues including threonine 277 and serine 281 within the activation loop of the kinase domain.

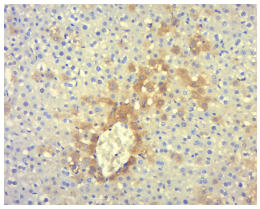
Applications: **IHC-P** (1:100-500)
IHC-F (1:100-500)
IF (1:100-500)
Flow-Cyt (1ug/Test)

Reactivity: Human, Mouse, Rat (predicted: Rabbit, Pig, Cow, Dog, GuineaPig)

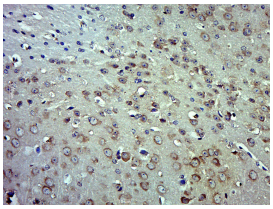
Predicted MW.: 93 kDa

Subcellular Location: Cytoplasm

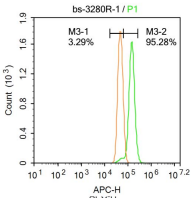
VALIDATION IMAGES



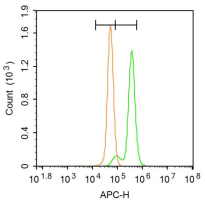
Paraformaldehyde-fixed, paraffin embedded (rat liver); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes; Blocking buffer (normal goat serum) at 37°C for 30min; Antibody incubation with (Phospho-MLK3(Thr277 + Ser281)) Polyclonal Antibody, Unconjugated (bs-3280R) at 1:400 overnight at 4°C, followed by a conjugated secondary (sp-0023) for 20 minutes and DAB staining.



Paraformaldehyde-fixed, paraffin embedded (Mouse brain); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes; Blocking buffer (normal goat serum) at 37°C for 30min; Antibody incubation with (Phospho-MLK3(Thr277 + Ser281)) Polyclonal Antibody, Unconjugated (bs-3280R) at 1:500 overnight at 4°C, followed by a conjugated secondary (sp-0023) for 20 minutes and DAB staining.



Blank control (Black line): A431 (Black). Primary Antibody (green line): Rabbit Anti-MLK3 antibody (bs-3280R) Dilution: 1µg /10^6 cells; Isotype Control Antibody (orange line): Rabbit IgG . Secondary Antibody (white blue line): Goat anti-rabbit IgG-AF647 Dilution: 1µg /test. Protocol The cells were fixed with 4% PFA (10min at room temperature)and then permeabilized with 0.1% PBST for 20 min at room temperature. The cells were then incubated in 5%BSA to block non-specific protein-protein interactions for 30 min at room temperature .Cells stained with Primary Antibody for 30 min at room temperature. The secondary antibody used for 40 min at room temperature. Acquisition of 20,000 events was performed.



Blank control: A431. Primary Antibody (green line): Rabbit Anti-MLK3(Thr277 + Ser281) antibody (bs-3280R) Dilution: 3µg /10^6 cells; Isotype Control Antibody (orange line): Rabbit IgG . Secondary Antibody: Goat anti-rabbit IgG-AF647 Dilution: 3µg /test. Protocol The cells were fixed with 4% PFA (10min at room temperature)and then permeabilized with 0.1% PBST for 20 min at room temperature. The cells were then incubated in 5%BSA to block non-specific protein-protein interactions for 30 min at room temperature .Cells stained with Primary Antibody for 30 min at room temperature. The secondary antibody used for 40 min at room temperature. Acquisition of 20,000 events was performed.