bs-5216R **[Primary Antibody]**

phospho-Bad (Ser118) Rabbit pAb

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DATASHEET

Host: Rabbit **Isotype:** IgG

Clonality: Polyclonal

GeneID: 572 **SWISS:** Q92934

Target: Bad (Ser118)

Immunogen: KLH conjugated Synthesised phosphopeptide derived from human BAD around the phosphorylation site of Ser118: RM(p-S)DE.

Purification: affinity purified by Protein G

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: Bad is a member of the Bcl2 family and acts to promote apoptosis by forming heterodimers with the survival proteins Bcl2 and BclxL, thus preventing them from binding with BAX. Bad is found on the outer mitochondrial membrane and, once phosphorylated in response to growth stimuli, translocates to the cytoplasm. The phosphorylation status of Bad represents a key checkpoint for death or cell survival. JNK-induced phosphorylation of BAD serine 128 promotes the apoptotic role of Bad by opposing the inhibitory effect of growth factor on Bad-mediated apoptosis. Cdc2-induced phosphorylation of Bad serine 128 has an inhibitory effect on its interaction with 14-3-3 proteins. The latter interaction is critical for Bad phosphorylation at serine 155, a site within the BH3 domain that leads to the release of BclxL and the promotion of cell survival. Alternative splicing of this gene results in two transcript variants which encode the same isoform.

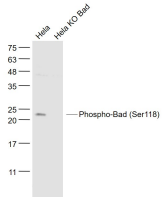
Applications: **WB** (1:500-2000)
IHC-P (1:100-500)
IHC-F (1:100-500)
IF (1:100-500)
Flow-Cyt (2ug/Test)

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

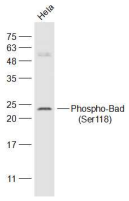
Predicted MW.: 22 kDa

Subcellular Location: Cell membrane ,Cytoplasm

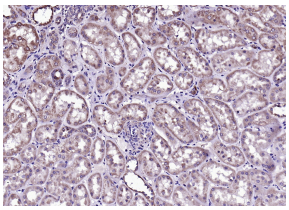
VALIDATION IMAGES



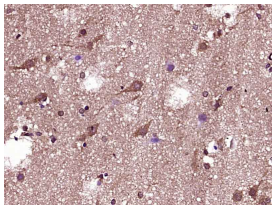
Sample: Hela(Human) Cell Lysate at 30 ug Hela KO Bad (Human) Cell Lysate at 30 ug Primary: Anti- Phospho-Bad (Ser118) (bs-5216R) at 1/1000 dilution Secondary: IRDye800CW Goat Anti-Rabbit IgG at 1/20000 dilution Predicted band size: 22 kD Observed band size: 22 kD

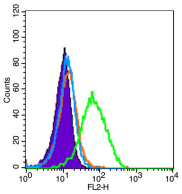


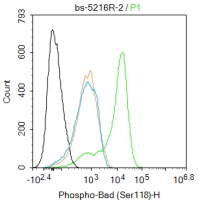
Sample: Hela(Human) Cell Lysate at 30 ug Primary: Anti-Phospho-Bad (Ser118) (bs-5216R) at 1/500 dilution Secondary: IRDye800CW Goat Anti-Rabbit IgG at 1/20000 dilution Predicted band size: 22 kD Observed band size: 22 kD



Paraformaldehyde-fixed, paraffin embedded (Human kidney); 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-Bad (Ser118)) Polyclonal Antibody, Unconjugated (bs-5216R) at 1:200 overnight at 4°C, followed by operating according to SP Kit(Rabbit) (sp-0023) instructionsand DAB staining.







Paraformaldehyde-fixed, paraffin embedded (Human brain glioma); 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-Bad (Ser118)) Polyclonal Antibody, Unconjugated (bs-5216R) at 1:400 overnight at 4°C, followed by operating according to SP Kit(Rabbit) (sp-0023) instructionsand DAB staining.

Blank control (Black line): Jurkat (Black). Primary Antibody (green line): Rabbit Anti-Bad(Ser118) antibody (bs-5216R) Dilution: 3µg /10^6 cells; Isotype Control Antibody (orange line): Rabbit IgG . Secondary Antibody (white blue line): Goat anti-rabbit IgG-PE Dilution: 1µg /test. Protocol The cells were fixed with 4% PFA (10min at room temperature)and then permeabilized with 0.1% PBS-Tween for 20 min at room temperature. The cells were then incubated in 5%BSA to block non-specific protein-protein interactions for 15 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:Jurkat. Primary Antibody (green line): Rabbit Anti-Phospho-Bad (Ser118) antibody (bs-5216R) Dilution: 2ug/Test; Secondary Antibody : Goat anti-rabbit IgG-FITC Dilution: 0.5ug/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.