bs-5182R **[Primary Antibody]**

phospho-AKT1 + AKT2 + AKT3 (Thr308+Thr309+Thr305) Rabbit pAb

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DATASHEET

Host: Rabbit **Isotype:** IgG

Clonality: Polyclonal

GeneID: 207 **SWISS:** P31749

Target: AKT1 + AKT2 + AKT3 (Thr308+Thr309+Thr305)

Immunogen: KLH conjugated Synthesised phosphopeptide derived from human AKT1 around the phosphorylation site of Thr308: MK(p-T)FC.

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: This gene encodes one of the three members of the human AKT serine-threonine protein kinase family which are often referred to as protein kinase B alpha, beta, and gamma. These highly similar AKT proteins all have an N-terminal pleckstrin homology domain, a serine/threonine-specific kinase domain and a C-terminal regulatory domain. These proteins are phosphorylated by phosphoinositide 3-kinase (PI3K). AKT/PI3K forms a key component of many signalling pathways that involve the binding of membrane-bound ligands such as receptor tyrosine kinases, G-protein coupled receptors, and integrin-linked kinase. These AKT proteins therefore regulate a wide variety of cellular functions including cell proliferation, survival, metabolism, and angiogenesis in both normal and malignant cells. AKT proteins are recruited to the cell membrane by phosphatidylinositol 3,4,5-trisphosphate (PIP3) after phosphorylation of phosphatidylinositol 4,5-bisphosphate (PIP2) by PI3K. Subsequent phosphorylation of both threonine residue 308 and serine residue 473 is required for full activation of the AKT1 protein encoded by this gene. Phosphorylation of additional residues also occurs, for example, in response to insulin growth factor-1 and epidermal growth factor. Protein phosphatases act as negative regulators of AKT proteins by dephosphorylating AKT or PIP3. The PI3K/AKT signalling pathway is crucial for tumor cell survival. Survival factors can suppress apoptosis in a transcription-independent manner by activating AKT1 which then phosphorylates and inactivates components of the apoptotic machinery. AKT proteins also participate in the mammalian target of rapamycin (mTOR) signalling pathway which controls the assembly of the eukaryotic translation initiation factor 4F (eIF4E) complex and this pathway, in addition to responding to extracellular signals from growth factors and cytokines, is disregulated in many cancers. Mutations in this gene are associated with multiple types of cancer and excessive tissue growth including Proteus syndrome and Cowden syndrome 6, and breast, colorectal, and ovarian cancers. Multiple alternatively spliced transcript variants have been found for this gene. [provided by RefSeq, Jul 2020]

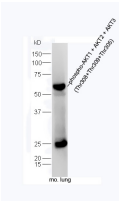
Applications: **WB** (1:500-2000)
IHC-P (1:100-500)
IHC-F (1:100-500)
IF (1:100-500)
Flow-Cyt (1μg/Test)
ICC/IF (1:100)

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

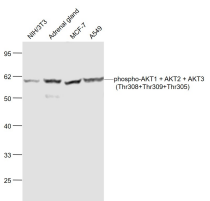
Predicted MW.: 53 kDa

Subcellular Location: Cell membrane ,Cytoplasm ,Nucleus

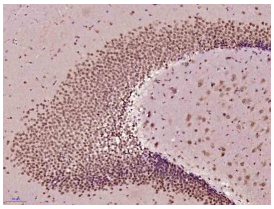
VALIDATION IMAGES



Sample: Lung (Mouse) Lysate at 40 ug Primary:



Sample: NIH/3T3(Mouse) Cell Lysate at 30 ug

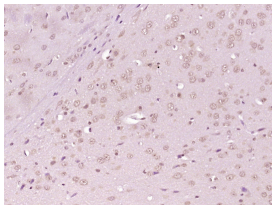


Paraformaldehyde-fixed, paraffin embedded

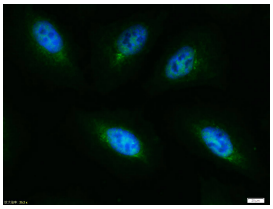
Anti-phospho-AKT1 + AKT2 + AKT3 (Thr308+Thr309+Thr305) (bs-5182R) at 1/300 dilution Secondary: HRP conjugated Goat-Anti-rabbit IgG (bs-0295G-HRP) at 1/5000 dilution Predicted band size: 53 kD Observed band size: 53 kD

Adrenal gland(Mouse) Lysate at 40 ug MCF-7(Human) Cell Lysate at 30 ug A549(Human) Cell Lysate at 30 ug Primary: Anti-phospho-AKT1+AKT2+AKT3 (Thr308+Thr309+Thr305) (bs-5182R) at 1/1000 dilution Secondary: IRDye800CW Goat Anti-Rabbit IgG at 1/20000 dilution Predicted band size: 60 kD Observed band size: 60 kD

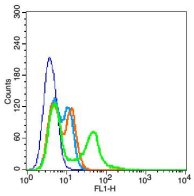
(Mouse brain); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes; Blocking buffer (normal goat serum) at 37°C for 30min; Antibody incubation with (phospho-AKT1 + AKT2 + AKT3 (Thr308+Thr309+Thr305)) Polyclonal Antibody, Unconjugated (bs-5182R) at 1:400 overnight at 4°C, followed by operating according to SP Kit(Rabbit) (sp-0023) instructionsand DAB staining.



Paraformaldehyde-fixed, paraffin embedded (Rat 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-AKT1 + AKT2 + AKT3 (Thr308+Thr309+Thr305)) Polyclonal Antibody, Unconjugated (bs-5182R) at 1:400 overnight at 4°C, followed by operating according to SP Kit(Rabbit) (sp-0023) instructionsand DAB staining.Antibody, Unconjugated(bs-5182R) 1:200, overnight at 4°C, followed by conjugation to the secondary antibody(SP-0023) and DAB(C-0010) staining



Tissue/cell: A549 cell; 4% Paraformaldehyde-fixed; Triton X-100 at room temperature for 20 min; Blocking buffer (normal goat serum, C-0005) at 37°C for 20 min; Antibody incubation with (phospho-AKT1 + AKT2 + AKT3 (Thr308+Thr309+Thr305)) polyclonal Antibody, Unconjugated (bs-5182R) 1:100, 90 minutes at 37°C; followed by a FITC conjugated Goat Anti-Rabbit IgG antibody at 37°C for 90 minutes, DAPI (blue, C02-04002) was used to stain the cell nuclei.



Blank control(blue):EC9706 (fixed with 2% paraformaldehyde for 10 min at 37°C). Primary Antibody:Rabbit Anti-phospho-AKT1 + AKT2 + AKT3 (Thr308+Thr309+Thr305) antibody (bs-5182R,Green); Dilution: 1μg in 100 μL 1X PBS containing 0.5% BSA; Isotype Control Antibody: Rabbit IgG(orange) ,used under the same conditions; Secondary Antibody: Goat anti-rabbit IgG-FITC(white blue), Dilution: 1:200 in 1 X PBS containing 0.5% BSA.

SELECTED CITATIONS

- **[IF=6.166]** Dawoon Jeong. et al. ELOVL2: a novel tumor suppressor attenuating tamoxifen resistance in breast cancer. Am J Cancer Res. 2021; 11(6): 2568–2589 WB ;Human. 34249416
- **[IF=6.014]** Lucca LE et al. TIGIT signaling restores suppressor function of Th1 Tregs. JCI Insight. 2019 Feb 7;4(3). pii: 124427. FCM ;Human. 30728325
- **[IF=4.527]** Zhao HJ et al. Destruction of redox and mitochondrial dynamics co-contributes to programmed cell death in chicken kidney under arsenite or/and copper (II) exposure. Ecotoxicology and Environmental Safety, 2019 179, 167–174. WB ;Chicken. doi:10.1016/j.ecoenv.2019.04.062
- **[IF=4.658]** Wang Y et al. Aspirin inhibits adipogenesis of tendon stem cells and lipids accumulation in rat injury tendon through regulating PTEN/PI3K/AKT signalling. J Cell Mol Med. 2019 Nov;23(11):7535-7544. WB ;Rat. 31557405
- **[IF=4.556]** Li Hao. et al. Suppressive Role of Bam32/DAPP1 in Chemokine-Induced Neutrophil Recruitment. Int J Mol Sci. 2021 Jan;22(4):1825 WB ;Mouse. 33673180