Product Datasheet

AKT1 [p Ser473] Antibody (17F6.B11) - BSA Free
NBP1-69923

Unit Size: 0.1 mg

Store at -20C. Avoid freeze-thaw cycles.

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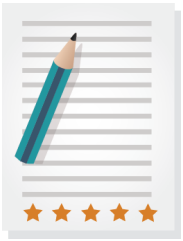
Updated 2/21/2025 v.20.1



NBP1-69923

AKT1 [p Ser473] Antibody (17F6.B11) - BSA Free

Product Information

Unit Size	0.1 mg
Concentration	Please see the vial label for concentration. If unlisted please contact technical services.
Storage	Store at -20C. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	17F6.B11
Preservative	0.01% Sodium Azide
Isotype	IgG1 Kappa
Purity	Protein A purified
Buffer	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2

Product Description

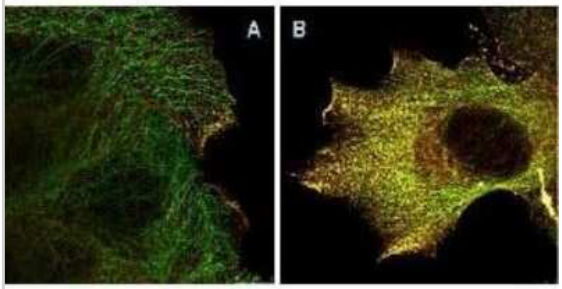
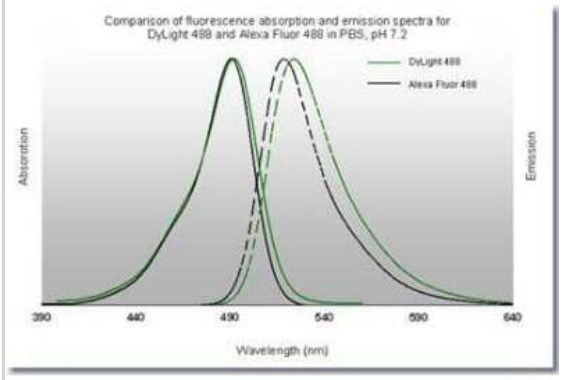
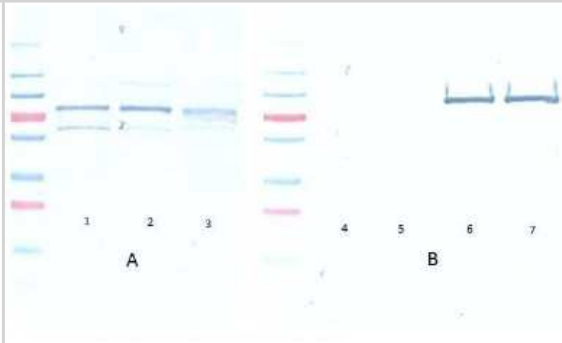
Description	This antibody Monoclonal Antibody was purified from concentrated tissue culture supernate by Protein A chromatography Store this antibody at -20C prior to opening. Aliquot contents and freeze at -20C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4C as an undiluted liquid. Dilute only prior to immediate use.
Host	Mouse
Gene ID	207
Gene Symbol	AKT1
Species	Human, Mouse, Rat, Monkey
Reactivity Notes	A BLAST analysis was used to suggest cross-reactivity with AKT1 pS473 from human, mouse, rat and chimpanzee sources based on 100% homology with the immunizing sequence. Cross-reactivity with AKT1 from other sources has not been determined. Cross-reactivity with AKT2 and AKT3 has not been determined.
Specificity/Sensitivity	This phospho specific monoclonal antibody is specific for phosphorylated human and mouse AKT protein at S473. A BLAST analysis was used to suggest cross-reactivity with AKT pS473 from human, mouse, rat and chimpanzee sources based on 100% homology with the immunizing sequence. Cross-reactivity with AKT from other sources has not been determined. Cross-reactivity with AKT2 and AKT3 has not been determined.
Immunogen	AKT1 [p Ser473] Antibody (17F6.B11) was produced by repeated immunizations with a synthetic peptide corresponding to residues surrounding S473 of human AKT11 protein, followed by hybridoma development. (Uniprot: P31749)

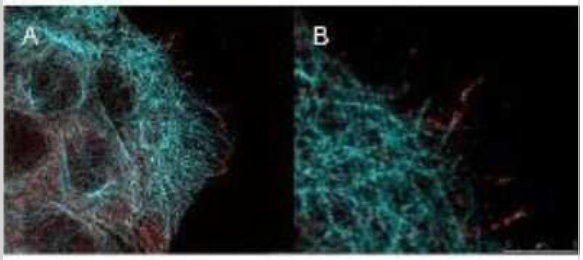
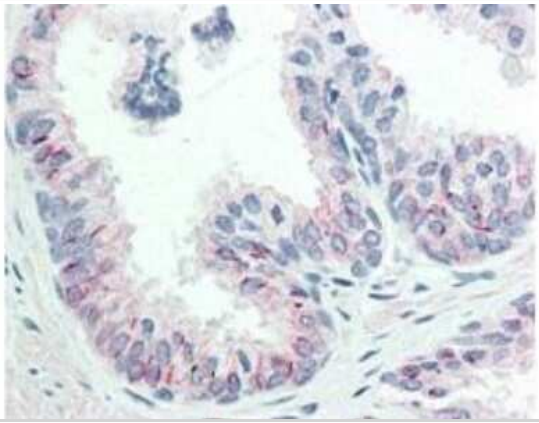
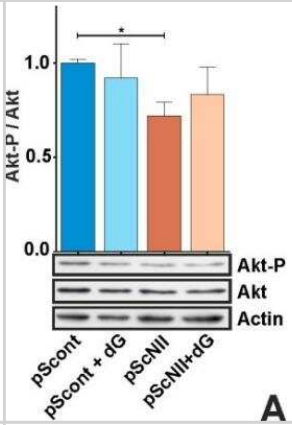
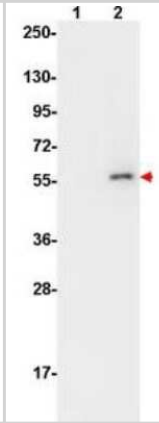
Product Application Details

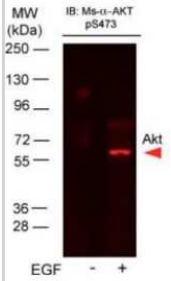
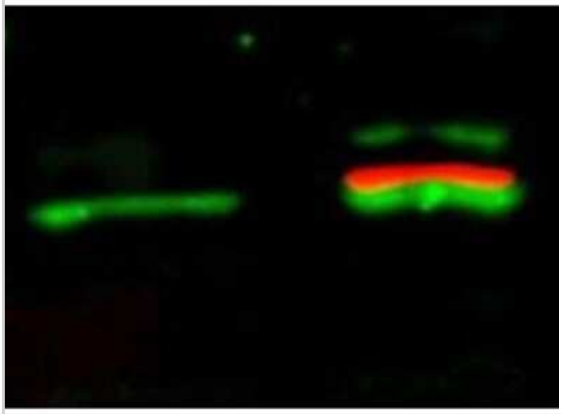
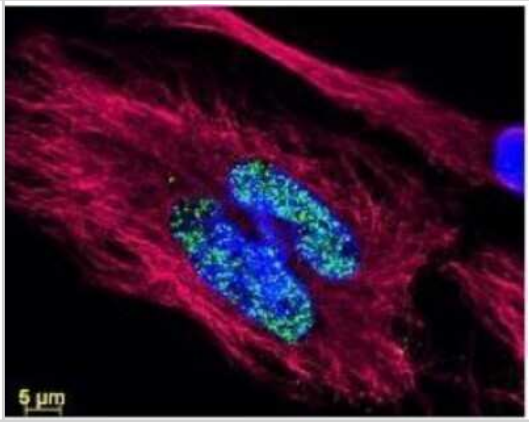
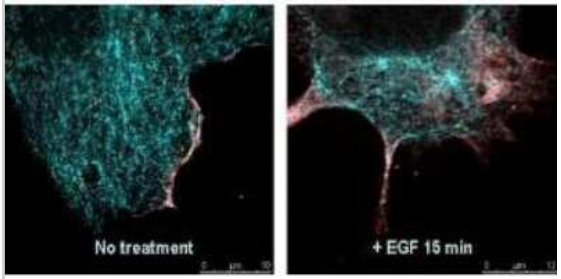
Applications	Western Blot, Dot Blot, ELISA, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation
Recommended Dilutions	Western Blot 1:500-1:3000, Flow Cytometry 1:10-1:1000, ELISA 1:20000, Immunohistochemistry 20 ug/ml, Immunocytochemistry/ Immunofluorescence 1:500-1:3000, Immunoprecipitation, Immunohistochemistry-Paraffin 1:10-1:500, Dot Blot

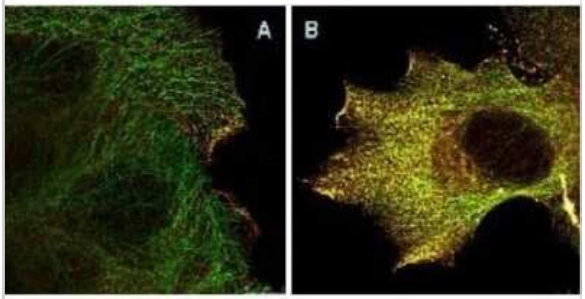
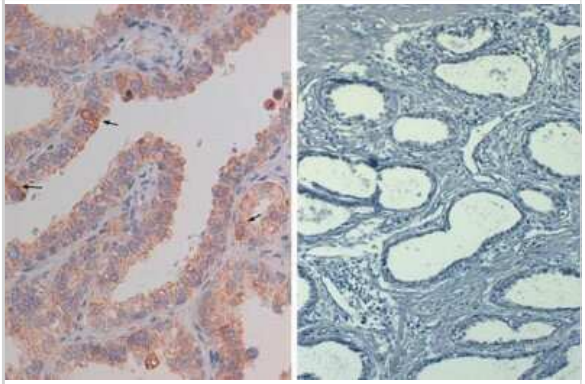
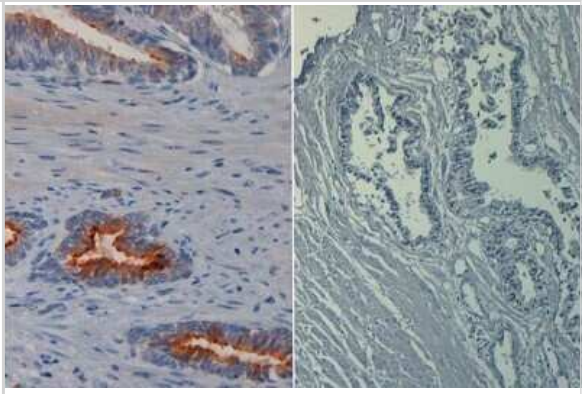
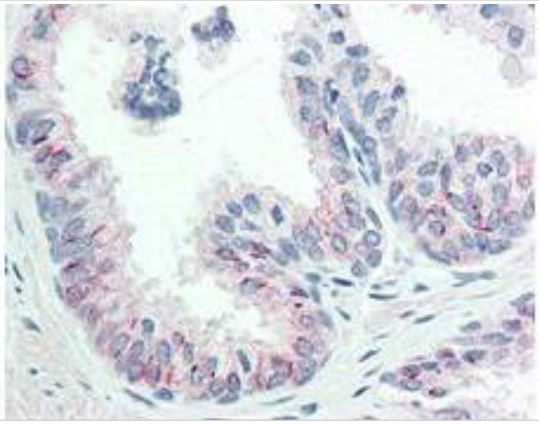
Application Notes	This product is tested in ELISA, immunofluorescence, immunohistochemistry, and western blotting. Expect a band approximately 56 kDa in size corresponding to phosphorylated AKT protein by western blotting in the appropriate cell lysate or extract. This phospho-specific monoclonal antibody reacts with human and mouse AKT pS473 and shows minimal reactivity by ELISA against the non-phosphorylated form of the immunizing peptide. Specific conditions for reactivity should be optimized by the end user. For immunohistochemistry use formalin-fixed paraffin-embedded sections. No pre-treatment of sample is required.

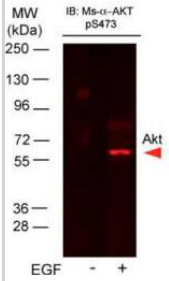
Images

Immunocytochemistry/Immunofluorescence: AKT1 [p Ser473] Antibody (17F6.B11) [NBP1-69923] - A431 cells. Panel A: serum starved, unstimulated cells. Panel B: serum starved, EGF stimulated for 15 mins. A massive increase in AKT-pS473 activation, as measured by intensity signal, peaked at 15 minutes and was associated with depolymerized tubulin. Panel A shows STED data (AKT-pS473, red channel) collected simultaneously with confocal signal (alpha-tubulin, green channel). Upon stimulation of cells with EGF, a rapid activation of AKT is observed (Panel B) along with a coincident change in the tubulin organization (yellow signal), as well as an extensive cell shape-change (cell membrane folding) and accumulation of AKT pS473 at the cell periphery.	
Flow Cytometry: AKT1 [p Ser473] Antibody (17F6.B11) [NBP1-69923] - Analysis using the DyLight 488 conjugate of AKT1 phospho Ser473 antibody. Image shows anti-histone detection using a DyLight 488 conjugate (green). Anti-Tubulin was detected using a DyLight 549 conjugate (red). Nuclei were counter-stained using DAPI (blue).	
Western Blot: AKT1 [p Ser473] Antibody (17F6.B11) [NBP1-69923] - Western Blot of AKT1 [p Ser473] antibody (17F6.B11). A: Lane 1) PDGF stimulated NIH 3T3 cells [10ul]. Lane 2) NIH 3T3 cells [10ul]. Lane 3) Hela whole cell lysate [10ul] (weak signal). B: Lane 4) GST negative control protein [100ng]. Lane 5) GST negative control protein [25ng]. Lane 6) AKT 1 recombinant protein [100ng]. Lane 7) AKT 1 recombinant protein [25ng]. Block: 5% BSA overnight at 4C. Primary antibody: Monoclonal anti-AKT antibody used at 1:1000 for overnight at 4C. Secondary antibody: HRP Conjugated goat anti-mouse 1:25K for 45 min at RT. Detection: TMB for 20 minutes, rinsed with deionized water, dried and scanned on conventional flatbed scanner.	

Immunocytochemistry/Immunofluorescence: AKT1 [p Ser473] Antibody (17F6.B11) [NBP1-69923] - High resolution STED immunofluorescence nanoscopy of AKT11 [p Ser473] antibody (17F6.B11). Tissue: A431 cells. The merge images (A) and at high magnification (B) show phosphorylated AKT1 colocalized with the distal microtubules. Fixation: 4% paraformaldehyde for 5 min and after washes blocked with 10% NGS/0.2% Triton X-100 for 30 min. Antigen retrieval: serum deprivation for 12 h. Primary antibody: AKT1 pS473 antibody at 10 ug/mL and alpha-tubulin (cyan) at 1.4 ug/mL for 1 h at RT. Secondary antibody: Atto 647N anti-Mouse IgG (ATTO TEC GmbH), and DyLight(TM)488 anti-Rabbit IgG were used at 1.0 ug/mL for 1h at RT for indirect detection. Localization: AKT1 pS473 is in the cytoplasm and also organized at the periphery of the cell. Staining: AKT1 pS473 as red signal with bis-benzimide (blue) nuclear counterstain.	
Immunohistochemistry: AKT1 [p Ser473] Antibody (17F6.B11) [NBP1-69923] - Immunohistochemistry of AKT11 [p Ser473] antibody (17F6.B11). Tissue: human prostate tissue. Fixation: formalin fixed paraffin embedded. Antigen retrieval: not required. Primary antibody: AKT1 pS473 antibody at 20 ug/mL for 1 h at RT. Secondary antibody: Dako's Techmate streptavidin-biotin reagents at 1:10,000 for 45 min at RT. Localization: AKT1 pS473 is nuclear and occasionally cytoplasmic. Staining: AKT1 pS473 as precipitated red signal with hematoxylin purple nuclear counterstain.	
Western Blot: AKT1 [p Ser473] Antibody (17F6.B11) [NBP1-69923] - Immunoblotting analysis. Representative images of the immunoblot analysis for (A) Akt, (B) p53 (C) AMPK, and (D) LC3 at 24 h of incubation with or without 20 mM dG are shown. beta-actin was used as loading control. Densitometry analysis was performed and the Akt-P/Akt ratio was calculated. The results are the mean +/- SEM of three independent experiments. * p < 0.05, ** p < 0.01. Image collected and cropped by CiteAb from the following publication (https://www.mdpi.com/1422-0067/19/7/2115) licensed under a CC-BY license.	
Western Blot: AKT1 [p Ser473] Antibody (17F6.B11) [NBP1-69923] - Western Blot of AKT11 [p Ser473] antibody (17F6.B11). Lane 1: untreated NIH/3T3 cell lysates. Lane 2: detects phosphorylated AKT1 (indicated by arrowhead at ~56 kDa) on PDGF stimulated NIH/3T3 cell lysates. Load: 10 ug per lane. Primary antibody: AKT1 pS473 antibody at 1:10,000 in TBS with 0.05% Tween-20 with 1% BSA, for 1 h at 4 C. Secondary antibody: HRP conjugated Gt-a-Mouse IgG was used at a 1:20,000 dilution for 1 h at 4 C with FemtoMax(TM) enhanced chemiluminescent reagent.	

Western Blot: AKT1 [p Ser473] Antibody (17F6.B11) [NBP1-69923] - Western Blot of AKT11 [p Ser473] antibody (17F6.B11). Lane 1: A431 cells. Lane 2: A431 cells stimulated for 15 min with EGF. Load: 35 ug per lane. Primary antibody: AKT1pS473 antibody at 1:400 for overnight at 4C. Secondary antibody: DyLight(TM)649 Conjugated Anti-AKT1 pS473 Monoclonal Antibody at 1:10,000 for 45 min at RT. Block: Blocking Buffer for Fluorescent Western Blotting overnight at 4C. Predicted/Observed size: 56kDa. Other band(s): none.	
Western Blot: AKT1 [p Ser473] Antibody (17F6.B11) [NBP1-69923] - Western Blot of AKT1 [p Ser473] antibody (17F6.B11). Lane 1: unstimulated NIH/3T3 lysates contain inactive unphosphorylated AKT11, green band. Lane 2: PDGF stimulated NIH/3T3 lysate contains both inactive (green band) and activated phosphorylated AKT11 (red band). Load: 10 ug per lane. Primary antibody: rabbit anti-AKT1 (pan) and mouse anti-AKT1 pS473 specific antibodies at 1:400 for overnight at 4C. Secondary antibody: DyLight(TM) 549 conjugated anti-rabbit IgG (green) and DyLight(TM) 649 conjugated anti-mouse IgG (red) secondary antibodies at 1:10,000 for 45 min at RT. Block: 5% BLOTTO overnight at 4C.	
Immunocytochemistry/Immunofluorescence: AKT1 [p Ser473] Antibody (17F6.B11) [NBP1-69923] - Analysis of DyLight 488 conjugate of NBP1-69923. This image shows anti-histone detection using a DyLight 488 conjugate (green). Anti-Tubulin was detected using a DyLight 549 conjugate (red). Nuclei were counter-stained using DAPI (blue).	
Immunocytochemistry/Immunofluorescence: AKT1 [p Ser473] Antibody (17F6.B11) [NBP1-69923]	

Immunocytochemistry/Immunofluorescence: AKT1 [p Ser473] Antibody (17F6.B11) [NBP1-69923] - Immunofluorescence Microscopy of AKT11 [p Ser473] antibody (17F6.B11) using STED nanoscopy to evaluate AKT1 activation and migration. Tissue: A431 cells. Antigen retrieval: Panel A: serum starved, unstimulated cells. Panel B: serum starved, EGF stimulated for 15 mins. A massive increase in AKT1-pS473 activation, as measured by intensity signal, peaked at 15 minutes and was associated with depolymerized tubulin. Staining: Panel A shows STED data (AKT1-pS473, red channel) collected simultaneously with confocal signal (a-tubulin, green channel). Upon stimulation of cells with EGF, a rapid activation of AKT1 is observed (Panel B) along with a coincident change in the tubulin organization (yellow signal), as well as an extensive cell shape-change (cell membrane folding) and accumulation of AKT1pS473 at the cell periphery.	
Immunohistochemistry-Paraffin: AKT1 [p Ser473] Antibody (17F6.B11) [NBP1-69923] - Analysis of Biotin conjugate of NBP1-69923. 20 ug/mL for 1 h at RT Secondary antibody: Streptavidin Conj. HRP 10 ug/ml Localization: nuclear and occasionally cytoplasmic Staining: antibody as precipitated red signal with a hematoxylin purple nuclear count	
Immunohistochemistry-Paraffin: AKT1 [p Ser473] Antibody (17F6.B11) [NBP1-69923] - Analysis of FFPE prostate tissue using AKT1 phospho Ser473 antibody (left panel). Negative control (right panel). Antigen retrieval with heat and pressure in citrate buffer pH 6.2. AKT1 phospho Ser473 antibody at 20 ug/mL. Secondary antibody Streptavidin-HRP at 10 ug/mL. Hematoxylin nuclear counterstain (purple). Image using the Biotin format of this antibody.	
Immunohistochemistry of Mouse anti-AKT pS473 antibody. Tissue: human prostate tissue. Fixation: formalin fixed paraffin embedded. Antigen retrieval: not required. Primary antibody: AKT pS473 antibody at 20 ug/mL for 1 h at RT. Secondary antibody: Dako's Techmate streptavidin-biotin reagents at 1:10,000 for 45 min at RT. Localization: AKT pS473 is nuclear and occasionally cytoplasmic. Staining: AKT pS473 as precipitated red signal with hematoxylin purple nuclear counterstain.	

Western Blot of Mouse Anti-AKTpS473 antibody. Lane 1: A431 cells. Lane 2: A431 cells stimulated for 15 min with EGF. Load: 35 ug per lane. Primary antibody: AKTpS473 antibody at 1:400 for overnight at 4C. Secondary antibody: DyLight(TM)649 Conjugated Anti-AKT1 [p Ser473] Antibody (17F6.B11) at 1:10,000 for 45 min at RT. Block: Blocking Buffer for Fluorescent Western Blotting overnight at 4C. Predicted/Observed size: 56kDa. Other band(s): none.	

Publications

Cevatemre B, Erk?sa M, Aztopal N et al. A promising natural product, pristimerin, results in cytotoxicity against breast cancer stem cells in vitro and xenografts in vivo through apoptosis and an incomplete autopaghy in breast cancer. Pharmacol Res 2017-12-01 [PMID: 29197639] (Mouse)

Pesi R, Petrotto E, Colombaioni L et al. Cytosolic 5'-Nucleotidase II Silencing in a Human Lung Carcinoma Cell Line Opposes Cancer Phenotype with a Concomitant Increase in p53 Phosphorylation Int J Mol Sci 2018-07-20 [PMID: 30037008] (WB, Human)

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NBP1-43319-0.5mg	Mouse IgG1 Kappa Isotype Control (P3.6.2.8.1)
NBP2-21678	AKT1 [p Ser473] Antibody (17F6.B11) [Biotin]

Limitations

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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