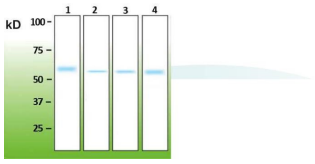
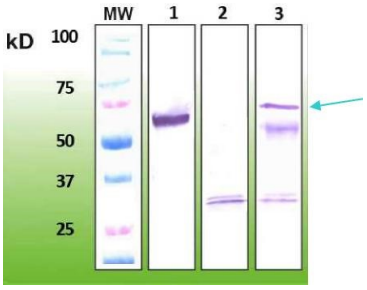
Product Data Sheet: AKT1

Cat. No:	AB-82322
Size:	100 ul
Clone:	Poly
Concentration:	1mg/ml
Host:	Rb
Isotype:	IgG
Immunogen:	Peptide derived from the C-terminal sequence of human AKT1 protein
Reactivity:	Hu, Ms, Rt
Applications:	Western blotting –1:1,000 – ELISA – 1:100,000 – 1:200,000
Purification:	Purified
Background:	Major clone of IgG obtained from the crude rabbit antiserum by in vitro cloning technology, detecting the Akt1 protein. This antibody cross-reacts with the Akt2 and Akt3 proteins at the lower signal intensity.
Form:	Liquid
Buffer:	20 mM Tris-HCl, pH 8.0
Storage:	10 µl aliquots at -20 °C.



Anti – Akt (AB82322)
Western blot analysis of Akt1 protein in mouse crude protein brain extract: lane 2 – 20 µg; lane 3 -100 µg; lane 4 – 200 µg of total protein loaded. Lane 1 represents the band corresponding to a 100ng of recombinant human Akt1



Immunoprecipitation-western blot analysis of Akt1 from mouse brain tissue
1. Recombinant Akt1 (50ng; positive control).
2. 200 µg of mouse brain protein extract, immuprecipitation protocol followed without the primary antibody (negative control).
Akt1 kinase immunoprecipitation from 200 µg of mouse brain protein extract with Akt1 antibody

IMMUNOPRECIPITAION PROTOCOL

1. 200 µg (57 µl) of protein sample from mouse crude brain extract (cell line protein crude extract or sample of biological fluid can be used alternatively) was centrifuged at 13,000xg, at 4°C, for 10 min.
2. The supernatant was carefully removed, and transferred to an Eppendorf tube.
3. For a lysate pre-clearing, 50 µl of Protein G-Sepharose beads were added, and incubated at 4°C, for 30 min.

4. After the separation of beads (700xg, 4°C, 2 min), the correction mix (20% of sample volume of 2.5% v/v Nonidet P-40, 5% w/v sodium deoxycholate, 0.5% w/v SDS) was added and gently mixed.
5. Polyclonal anti-Akt1 antibody (5 µl) was added to the sample, mixed, and incubated on ice for 1 hour.
6. During this time, the appropriate volume of Protein G-Sepharose (~50 µl of beads in 20% ethanol) was washed with 2×50 volumes of 20mM Tris,HCl, pH 7.5, and centrifuged at 700xg, 4°C, 2 min.
7. After 1 hour of incubation, the sample was added to the washed Protein G-sepharose beads, and the mixture was incubated at 4°C, for 1 hour.
8. The beads were consequently washed 3x with washing buffer (RIPA: 10mM Tris,HCl, pH 7.5, 140 mM NaCl, 1% v/v Nonidet P-40, 1% w/v sodium deoxycholate, 0.1% w/v SDS), and 1x with 10mM Tris.HCl, pH 7.5 (this wash removes the detergents, deoxycholate in particular, because its presence in sample may reduce the quality of SDS PAGE protein separation).
9. The beads were resuspended in the small volume of sample buffer (30-50 µl of 125mM Tris.HCl, pH
6.8; 3.3% SDS, 5% β-mercaptoethanol), and the immune complex was dissociated at 60°C for 5 min.
10. To the resulting supernatant (after centrifugation at 10,000xg for 2min), 10% v/v of glycerol containing 0.1% w/v of bromphenol blue was added, and the sample was boiled for 3 min.
11. Sample was applied to the SDS-PAGE and western blot performed with, anti-Akt1 primary antibody.

IMMUNOCYTOCHEMISTRY (ICC) PROTOCOL

1. Coat coverslips with 1% gelatin-coating solution for 2 hours at room temperature (RT); rinse with distilled water, and let to dry overnight. Before plating the cells, wash the coated coverslips briefly with PBS.
2. Fix the cells with 4% paraformaldehyde solution (in PBS, pH 7.2), for 15 min at RT.
3. Wash 2 x 3 min with PBS.
4. Permeabilize the cells with 0.1% Triton X-100 solution (in PBS, pH 7.2) for 5 min on ice.
5. Wash 2 x 3 min with PBS.
6. Incubate the cells in blocking buffer (0.3M glycine in PBS, 2% BSA) for 30 min at RT.
7. Incubate the cells with primary antibody: anti-Akt1 clonal antibody at the dilution of 1:100 – 1:400 in antibody dilution buffer (PBS, 1% BSA) for 1 hour at RT in humid chamber.
8. Wash 2 x 3 min with PBS.
9. Apply the secondary antibody (in this case, the goat anti-rabbit IgG-FITC IS was used at 1:300 in antibody dilution buffer, and cells were incubated for 1 hour at RT in dark).
10. Wash 3 x 3 min with PBS.
11. Rinse once with distilled water.
12. Mount the slide for observation, with a drop of anti-fade mounting medium.