

Novocastra™ Liquid Mouse Monoclonal Antibody Akt (phosphorylated)

Product Code: NCL-L-Akt-Phos

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| Intended Use | FOR RESEARCH USE ONLY. |
| Specificity | Human Akt phosphorylated at Ser473 |
| Clone | LP18 |
| Ig Class | IgG2b Kappa |
| Antigen Used for Immunizations | A chemically synthesized phosphorylated peptide corresponding to residues around Ser473 of the human Akt (phosphorylated) molecule. |
| Hybridoma Partner | Mouse myeloma (p3-NS1-Ag4.1) |
| Preparation | Liquid tissue culture supernatant containing 15 mM sodium azide. Volume as indicated on vial label. |
| Effective on Frozen Tissue | Not evaluated. |
| Effective on Paraffin Wax Embedded Tissue | Yes (using heat induced epitope retrieval with citrate-based buffer, pH 6.0: see overleaf) |
| Recommendations on Use | Immunohistochemistry: Typical working dilution 1:50. Heat induced epitope retrieval technique using Citrate-based buffer, pH 6.0. 30 minutes primary antibody incubation at 25 °C. Polymer detection recommended. Technical note: NCL-L-Akt-Phos is not recommended for use with PBS, since the use of PBS-based wash buffers and possibly PBS-based antibody diluents gives increased background staining and decreased staining intensity. |
| Positive Controls | Immunohistochemistry: Skin |
| Staining Pattern | Nuclear and Cytoplasmic |
| Storage and Stability | Store liquid antibody at 4 °C. Under these conditions, there is no significant loss in product performance up to the expiry date indicated on the vial label. Prepare working dilutions on the day of use. |
| General Overview | Akt-1, also referred to as Protein Kinase B (PKB) or Rac alpha is a member of the Akt serine/ threonine protein kinase family. It plays an important role in many biological responses including metabolism, cell survival and growth by phosphorylating and inactivating several targets including GSK 3 beta, caspase 9, BAD and the Forkhead transcription factor. |
| General References | Brazil D, Yang Z and Hemmings B. Trends in Biochemical sciences. 29(5):233–242 (2004). Nicholson K and Anderson N. Cellular Signalling. 14:381–395 (2002). Lawlor M and Alessi D. Journal of Cell Science. 114: 2903–2910 (2001). |



Instructions for Use

Heat Induced Epitope Retrieval Combined With Polymer Detection For Immunohistochemical Demonstration On Paraffin Sections

1. Cut and mount sections on slides coated with a suitable tissue adhesive.
2. Deparaffinize sections and rehydrate to distilled water.
3. Place sections in 0.5% hydrogen peroxide/methanol for 10 minutes (or use other appropriate endogenous peroxidase blocking procedure). Wash sections in tap water.
4. Heat 1500 mL of the recommended epitope retrieval solution (Citrate based pH 6.0 - Epitope Retrieval Solution unless otherwise indicated overleaf) in a stainless steel pressure cooker until boiling. Cover but do not lock lid.
5. Position slides into metal staining racks (do not place slides close together as uneven staining may occur) and lower into pressure cooker ensuring slides are completely immersed in epitope retrieval solution. Lock lid.
6. When the pressure cooker reaches operating temperature and pressure (after about 5 minutes) start a timer for 1 minute (unless otherwise indicated on the data sheet).
7. When the timer rings, remove pressure cooker from heat source and run under cold water with lid on. **DO NOT OPEN LID UNTIL THE INDICATORS SHOW THAT PRESSURE HAS BEEN RELEASED.** Open lid, remove slides and place immediately into a bath of tap water.
8. Wash sections once using fresh Tris-Buffered Saline (TBS, pH 7.6) buffer for 5 minutes.
9. Place sections in diluted normal serum (eg NCL-G-SERUM) for 10 minutes.
10. Incubate sections with primary antibody.
11. Wash twice, each time using fresh TBS buffer for 5 minutes.
12. For visualization of the bound primary antibody, follow instructions supplied with the Polymer Detection System.
13. Counterstain with hematoxylin (if required), dehydrate and mount.

** (In most applications, Phosphate Buffered Saline, pH 7.6, can be used instead of TBS, pH 7.6).*

Safety Note

To ensure the correct and safe use of your pressure cooker, PLEASE READ THE MANUFACTURER'S INSTRUCTIONS.