Novocastra™ Liquid Mouse Monoclonal Antibody Aurora Kinase 2

Product Code: NCL-L-AK2

Intended Use	FOR RESEARCH USE ONLY.
Specificity	Human Aurora Kinase 2.
Clone	JLM28
Ig Class	IgG2a
Antigen Used for Immunizations	131 amino acid region of the N-terminus of human Aurora Kinase 2 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 1 mM EDTA (pH 8.0) unmasking solution combined with the high temperature antigen unmasking technique: see overleaf).
Recommendations on Use	Immunohistochemistry: Typical working dilution 1:50. High temperature antigen unmasking technique using 1 mM EDTA (pH 8.0). 60 minutes primary antibody incubation at 25 °C. Standard ABC technique. Western Blotting: Typical working dilution 1:500–1:1000 (ECL™, Amersham Pharmacia Biotech).
Positive Controls	Immunohistochemistry: Tonsil. Western Blotting: HeLa cell line.
Staining Pattern	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	Aurora Kinase 1 and 2 encode cell cycle-regulated serine/threonine kinases that are involved in microtubule spindle activities during mitosis and meiosis. Aurora Kinase 2, also known as STK15, BTAK, ARK1 and AIK, localizes to interphase and mitotic centrosomes and to the spindle poles. It is degraded rapidly after G2/M phase release in mammalian cells. Aurora Kinase 2 is expressed at high levels in testis and various proliferating cells, including HeLa cells. Aurora Kinase 2 is regulated by phosphorylation which is important both for its activity and stability. The inhibition of its activity leads to the formation of a monopolar spindle because its activity is necessary for centrosome separation. Aurora Kinase 2 overexpression leads to centrosome amplification, chromosome instability and transformation in mammalian cells. Overexpression of both active an inactive Aurora Kinase 2 can lead to polyploidy. This suggests that Aurora Kinase 2 can behave as a dominant negative mutant and inhibit other aurora kinases. When inactive kinase is expressed, however, the cells eventually die and do not become immortalized, unlike with the active kinase.
General References	Honda K, Mihara H, Kato Y, et al.. Oncogene. 19: 2812–2819 (2000). Walter A O, Seghezzi W, Korver W, et al.. Oncogene 19 (42): 4906–4916 (2000). Tanaka T, Kumura M, Matsunaga K, et al.. Cancer Research. 59 (9): 2041–2044 (1999). Bischoff J R, Anderson L, Zhu Y, et al.. EMBO Journal. 17 (11): 3052–3065 (1998).

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Instructions for Use

High Temperature Antigen Unmasking Technique 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 unmasking solution (0.01 M citrate buffer, pH 6.0 (or Epitope Retrieval Solution, RE7113) unless otherwise indicated overleaf) until boiling in a stainless steel pressure cooker. 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 unmasking 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 in TBS* buffer (pH 7.6) for 1 x 5 minutes.
9. Place sections in diluted normal serum (or RTU Normal Horse Serum) for 10 minutes.
10. Incubate sections with primary antibody. Use Antibody Diluent RE7133 (where available).
11. Wash in TBS buffer for 2 x 5 minutes.
12. Incubate sections in an appropriate biotinylated secondary antibody.
13. Wash in TBS buffer for 2 x 5 minutes.
14. Incubate slides in ABC reagent (or RTU streptavidin/peroxidase complex).
15. Wash in TBS buffer for 2 x 5 minutes.
16. Incubate slides in DAB or other suitable peroxidase substrate.
17. Wash thoroughly in running tap water.
18. Counterstain with hematoxylin (if required), dehydrate and mount.

Solutions

0.01 M CITRATE BUFFER (pH 6.0) or RE7113 (where available).
Add 3.84 g of citric acid (anhydrous) to 1.8 L of distilled water. Adjust to pH 6.0 using concentrated NaOH. Make up to 2 L with distilled water.

1 mM EDTA (pH 8.0) or RE7116 (where available).
Add 0.37 g of EDTA (SIGMA product code E-5134) to 1 litre of distilled water. Adjust pH to 8.0 using 1.0 M NaOH.

20 mM TRIS/ 0.65 mM EDTA/ 0.005% TWEEN (pH 9.0) or RE7119 (where available).
Dissolve 14.4 g Tris (BDH product code 271197K) and 1.44 g EDTA (SIGMA product code E-5134) to 0.55 L of distilled water. Adjust pH to 9.0 with 1 M HCl and add 0.3 mL Tween 20 (SIGMA product code P-1379). Make up to 0.6 L with distilled water. This is a 10x concentrate which should be diluted with distilled water as required (eg 150 mL diluted with 1350 mL of distilled water).

** In most applications, 10 mM phosphate, 0.15 M NaCl, pH 7.6 (PBS) can be used instead of 50 mM Tris, 0.15 M NaCl, pH 7.6 (TBS).*

Safety Note

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