Novocastra™ Lyophilized Rabbit Polyclonal Antibody Ki67 Antigen

Product Code: NCL-Ki67p

Intended Use	FOR RESEARCH USE ONLY.
Specificity	Human Ki67 nuclear antigen expressed in all proliferating cells during late G1, S, M and G2 phases of the cell cycle
Antigen Used for Immunizations	Prokaryotic recombinant fusion protein corresponding to a 1086 bp Ki67 motif-containing cDNA fragment.
Preparation	Lyophilized rabbit serum in PBS with 1% BSA containing 15 mM sodium azide. Reconstitute with the volume of sterile distilled water indicated on the vial label.
Effective on Frozen Tissue	Not evaluated.
Effective on Paraffin Wax Embedded Tissue	Yes (using the high temperature antigen unmasking technique: see overleaf).
Recommendations on Use	Immunohistochemistry: Typical working dilution 1:2000. Citrate-based buffer, pH 6.0. 30 minutes primary antibody incubation at 25 °C. Polymer detection recommended. Western Blotting: Not evaluated.
Positive Controls	Immunohistochemistry: Tonsil, thymus and oral mucosa.
Staining Pattern	Nuclear.
Storage and Stability	Store unopened lyophilized 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. The reconstituted antibody is stable for at least two months when stored at 4 °C. For long term storage, it is recommended that aliquots of the antibody are frozen at -20 °C (frost-free freezers are not recommended). Repeated freezing and thawing must be avoided. Prepare working dilutions on the day of use.
General Overview	The Ki67 antigen is a human nuclear protein which is expressed in all active parts of the cell cycle (G1, S, G2 and mitosis), but absent in resting cells (G0). In contrast to many other cell cycle-associated proteins, the Ki67 antigen is consistently absent in quiescent cells and is not detectable during DNA repair processes. Thus, the presence of Ki67 antigen is strictly associated with the cell cycle and confined to the nucleus, suggesting an important role of this structure in the maintenance and/or regulation of the cell division cycle.
General References	Cattoretti G and Fei Q. Antigen Retrieval Techniques. 165–179. Eds. Shi S-R, Gu J and Taylor C R. Eaton Publishing (2000). Key G, Becker M H G, Baron B, et al.. Laboratory Investigation. 68 (6): 629–636 (1993). McCormick D, Yu C, Hobbs C, et al.. Histopathology. 22: 543–547 (1993). Schlüeter C, Duchrow M, Wohlenberg C, et al.. The Journal of Cell Biology. 123 (1): 513–522 (1993). Gerdes J, Li L, Schlüeter C, et al.. American Journal of Pathology. 138 (4): 867–873 (1991). Gerdes J, Schwab U, Lemke H, et al.. International Journal of Cancer. 31: 13–20 (1983). Stefanini M, De Martino C and Zamboni L. Nature. 216: 173–174 (1967).

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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.