Novocastra™ Lyophilized Mouse Monoclonal Antibody Retinoblastoma Gene Protein

Product Code: NCL-RB

Intended Use	FOR RESEARCH USE ONLY.
Specificity	Human retinoblastoma gene protein (105kD)
Clone	1F8
Ig Class	IgG1
Antigen Used for Immunizations	Prokaryotic recombinant galactosidase fusion protein corresponding to the retinoblastoma gene protein.
Hybridoma Partner	Mouse myeloma (Sp2/0 Ag14).
Preparation	Lyophilized tissue culture supernatant containing 15 mM sodium azide. Reconstitute with 1 mL or 0.1 mL of sterile distilled water as indicated on vial label.
Effective on Frozen Tissue	Yes, optimum fixative, Zamboni's, 10 minutes at 25 °C (see Stefanini et al., 1967).
Effective on Paraffin Wax Embedded Tissue	Yes (using the high temperature antigen unmasking technique: see overleaf).
Recommendations on Use	Immunohistochemistry: Typical working dilution 1:20–1:50. High temperature antigen unmasking technique. 60 minutes primary antibody incubation at 25 °C. Standard ABC technique. Western Blotting: Typical working dilution 1:50–1:100.
Positive Controls	Immunohistochemistry: Generally, the researcher will be looking for loss of expression of retinoblastoma gene protein. It is likely that a high proportion of most types of tumor will show nuclear staining. Western Blotting: WI38 cell line.
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	Retinoblastoma (Rb) is a rare tumor of the retina, associated with mutations of chromosome 13. The nuclear phosphoprotein encoded by the Rb tumor suppressor gene is present in many cells and may indirectly regulate cell growth by activating the transcription factor ATF-2. Activation of ATF-2 initiates expression of TGF-beta2 which in turn inhibits transcription of genes affecting cell growth.
General References	Takes R P, Baatenburg de Jong R J, Wijffels K, et al.. Journal of Pathology. 194: 298–302 (2001). Bartek J, Vojtésék B, Grand R J A, et al.. Oncogene. 7: 101–108 (1992). Cowell J K. British Journal of Cancer. 63: 333–336 (1991). Sanders B M, Jay M, Draper G J, et al.. British Journal of Cancer. 60: 358–365 (1989). Varley J M, Armour J, Swallow J E, et al.. Oncogene. 4: 725–729 (1989). Harbour J W, Lai S L, Peng J W, et al.. Science. 241: 353–357 (1988). Lee W H, Shew J Y, Hong F D, et al.. Nature. 329: 642–645 (1987). Stefanini M, De Martino C and Zamboni L. Nature. 216: 173–174 (1967).

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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.