Novocastra™ Lyophilized Mouse Monoclonal Antibody Minichromosome Maintenance Protein 5

Product Code: NCL-MCM3

Intended Use
FOR RESEARCH USE ONLY.

Specificity
Human minichromosome maintenance protein 3

Clone
JCC07

Ig Class
IgG1, kappa

Antigen Used for Immunizations
Prokaryotic recombinant protein corresponding to 321 amino acids of the C-terminus of the human minichromosome maintenance protein 3 molecule.

Hybridoma Partner
Mouse myeloma (SP-2).

Preparation
Lyophilized tissue culture supernatant containing 15 mM sodium azide.
Reconstitute with the volume of sterile distilled water as indicated on vial label.

Effective on Frozen Tissue
No

Effective on Paraffin Wax Embedded Tissue
Yes (using the high temperature antigen unmasking technique: see overleaf).

Recommendations on Use
Immunohistochemistry: Typical working dilution 1:100–1:200. High temperature antigen unmasking technique. 60 minutes primary antibody incubation at 25 °C. Standard ABC technique. Western Blotting: Not recommended.

Positive Controls
Immunohistochemistry: Tonsil.

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
Minichromosome maintenance (MCM) proteins play an essential part in eukaryotic DNA replication. Each of the MCM proteins have DNA-dependent ATPase motifs in their central domain that are conserved from yeast to mammals. Both ATPase activity and helicase activity, which displaces oligonucleotides annealed to single-stranded circular DNA, are associated with an MCM protein complex. Levels of MCM proteins generally increase in a variable manner as normal cells progress from G0 into G1/S phase of the cell cycle. In the G0 phase, MCM2 and MCM5 proteins are much less abundant than the MCM7 and MCM3 proteins. MCM3 protein is detected in nuclei of cells in the proliferating zone of human epidermal tissue, but in decreasing amounts in nuclei of differentiating cells in the upper cell layers. MCM3 protein is also expressed in cells that have ceased to proliferate, but are not terminally differentiated.

General References
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.