



MONOCLONAL ANTIBODY

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Catalog NM-MA-003

Anti-gamma-Tubulin (E39) - Centrosome Marker -

BACKGROUND

Gamma-tubulins constitute a ubiquitous and highly conserved subfamily of the tubulin family. The protein is found at microtubule-organising centres, such as the spindle poles or the centrosome. It remains associated with the centrosome when microtubules are depolymerised, suggesting that it is an integral component that might play a role in minus-end nucleation of microtubule assembly. Thus, gamma-tubulins can be used for centrosome marker of the cells.

Product type	Primary antibody
Immunogen	Whole length of human gamma-tubulin 1 protein
Raised in	Mouse
Myeloma	-
Clone number	E39
Isotype	IgG1, λ
Source	The hybridoma was established by fusion of mouse myeloma cells with Balb/c mouse splenocytes immunized with whole length of human gamma-tubulin 1 protein. This hybridoma (Clone E39) culture supernatant was collected and precipitated with ice-cold ammonium sulfate. After centrifugation, the pellet dissolved in small volume of double-distilled water was dialysed against PBS. The dialysate was then lyophilized.
Purification	-
Form	This antibody is lyophilized form. Reconstitute with 100 μ l of distilled water. No preservative is contained.
Storage buffer	PBS, No preservative is contained.
Concentration	-
Volume	100 μ L
Label	Unlabeled
Specificity	Gamma-Tubulin
Cross reactivity	Human, Mouse, and Rat
Storage	Lyophilized form: store at -20 to -80 °C. Reconstituted form: store at -20 °C After reconstitution, it is stable for at least 1 year when stored at -20 °C. It should be divided into small quantity to avoid many freezing and thawing.
Other	UniProtKB/Swiss-Prot : (Human) P23258 , (Mouse) P83887 , (Rat) P83888

Application notes	
Recommended dilutions	<ul style="list-style-type: none">• Immunocytochemistry: 1/200• Western blotting: 1/1000• ELISA: 1/1000

Other applications have not been tested.
Optimal dilutions/concentrations should be determined by the end user.

References	-
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ANTIBODY CHARACTERIZATION

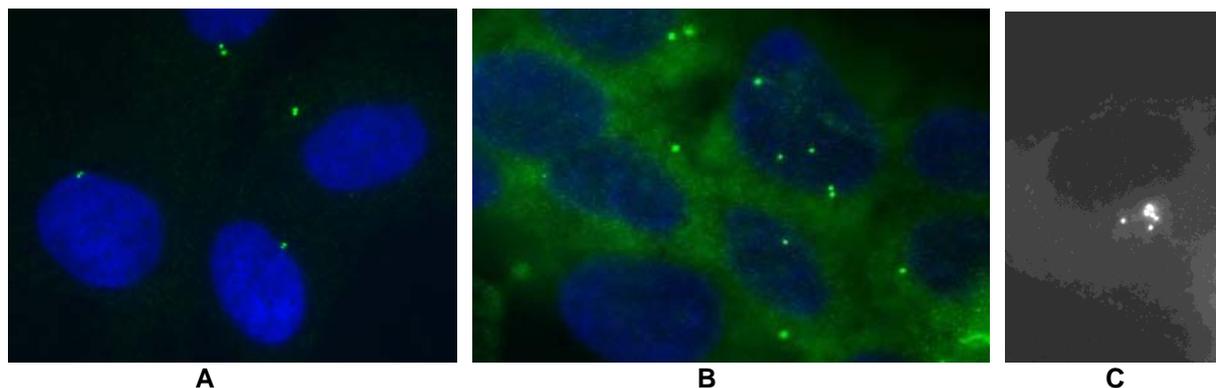


Figure 1 Monoclonal antibody (E39) recognizes gamma-tubulin located at the centrosomes. Gamma-tubulin in normal or cancer cells was stained by an immunofluorescence described below. The presence of supernumerary centrosomes is a common event in human tumors. Yellow and blue indicate gamma-tubulin and nuclear DNA, respectively.
A; normal human fibroblasts, **B;** colon cancer cell line (CaCo-2), **C;** fibrosarcoma cell line (HT-1080).

Immunofluorescence Protocol

- 1) Culture the cells in an appropriate condition in 35-mm glass-bottom dishes (MatTek, Ashland, MA). (For example, inoculate 3×10^5 cells per dish, then incubate for one or two days in a CO₂ incubator.)
- 2) Wash the cells 2 times with 2 mL of Dulbecco's PBS (DPBS).
- 3) Pour 2 mL of 100% cold MeOH into each dish, and fix the cells for 10 minutes on ice.
- 4) Dry the cells at room temperature.
- 5) Wash the cells 5 times with 2 mL of PBS-T (0.05% Tween-20 in PBS).
- 6) Add 70 μ L of 20% FBS in PBS to prevent non-specific antibody binding.
- 7) Incubate 30 minutes at 37 °C with gentle shaking.
- 8) Wash the cells 5 times with 2 mL of PBS-T.
- 9) Add 70 μ L of E39 antibodies diluted with PBS containing 5% FBS as suggested in the APPLICATIONS onto the cells and incubate for 30 minutes at 37 °C with shaking (Optimization of antibody concentration or incubation condition is recommended if necessary.)
- 10) Wash the cells 5 times with 2 mL of PBS-T.(Subsequent steps must be done in the dark.)
- 11) Add 70 μ L of 1:200 Alexa Fluor 488-F(ab')₂ fragment of anti-mouse IgG (H+L) (Molecular Probes, Cat. No. A-11020) diluted with PBS containing 5% FBS and incubate for 30 minutes at 37 °C with shaking.
- 12) Wash the cells 5 times with 2 mL of PBS-T.
- 13) To stain DNA, add 70 μ L of 0.05 μ g/mL DAPI in PBS and incubate for 5 minutes at room temperature with shaking.
- 14) Wash the cells 5 times with 2 mL of PBS-T.
- 15) Promptly add 20 μ L of Vectashield mounting medium (Vector, Cat. No. H-1000) onto the cells, then put a cover slip on them.

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