COSMO BIO CO., LTD.
Inspiration for Life Science

MONOCLONAL ANTIBODY

For research use only, Not for diagnostic use

Catalog NM-MA-003

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

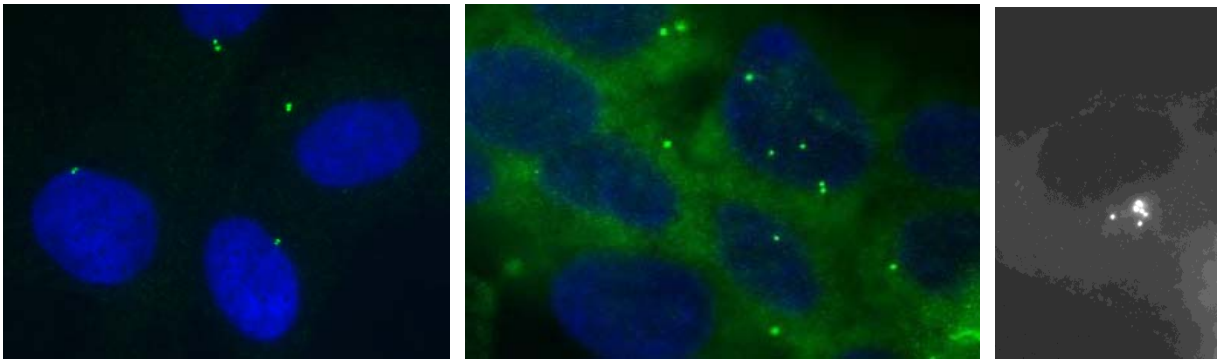
BACKGROUND

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 maker of the cells.

Product type	Primary antibody
Immunogen	Whole length of human gamma-tubulin 1 protein
Rased 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 µl of distilled water. No preservative is contained.
Strage buffer	PBS, No preservative is contained.
Concentration	-
Volume	100 µL
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**	• **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	-

ANTIBODY CHARACTERIZATION



A B C

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 $3x10^5$ cells per dish, then incubate for one or two days in a CO_2 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 µL 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 µL 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 µL 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 µL of 0.05 µg/ 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 µL of Vectashield mounting medium (Vector, Cat. No. H-1000) onto the cells, then put a cover slip on them.

For research use only, Not for diagnostic use.

COSMO BIO CO., LTD.
Inspiration for Life Science
TOYO 2CHOME, KOTO-KU, TOKYO, 135-0016, JAPAN
URL: http://www.cosmobio.co.jp e-mail: export@cosmobio.co.jp
[Outside Japan] Phone : +81-3-5632-9617 [国内連絡先] Phone : +81-3-5632-9610
FAX : +81-3-5632-9618 FAX : +81-3-5632-9619