

Data Sheet

## Anti- $\beta$ -Tubulin antibody, Mouse monoclonal

Clone D66, purified from hybridoma cell culture

**T0198**

### Product Description

Monoclonal Anti- $\beta$ -Tubulin (mouse IgG1 isotype) is derived from the D66 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from a BALB/c mouse immunized with a sea urchin (*Lytechinus pictus*) sperm axonemal protein.<sup>1</sup> The isotype is determined by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents, Cat. No. ISO2.

Monoclonal Anti- $\beta$ -Tubulin recognizes all mouse brain  $\beta$ -tubulin isoforms ( $\beta$  and  $\beta'$  subpopulations), and sea urchin  $\beta$  2-tubulin.<sup>1</sup> The epitope recognized by the antibody is localized in the C-terminal region of  $\beta$  -tubulin (amino acids 427-432 in sea urchin  $\beta$ 2-tubulin) which has been identified as important in flagellar motility.<sup>1</sup> The product is useful in ELISA,<sup>1</sup> immunoblotting,<sup>1</sup> (approximately 50 kDa), immunocytochemistry (3% paraformaldehyde-0.5% Triton® X-100 and methanol-acetone), and immunoprecipitation.<sup>2</sup> It is also useful for studies involving the inhibition of flagellar motility in permeabilized sperm models.<sup>1</sup> Species cross reactivity is observed with human,<sup>1</sup> monkey, bovine, dog, rabbit, hamster, rat, mouse, chicken, sea urchin (*Lytechinus pictus*,<sup>1,2</sup> and *Paracentrotus lividus*<sup>1</sup>), dinoflagellate (*Oxyrrhis marina*),<sup>1</sup> and *Chlamydomonas reinhardtii*.<sup>1</sup>

Many cellular functions are dependent on the proper organization of microtubules, since they are essential for mitosis, meiosis, some forms of organelle movement, intracellular transport, flagellar movement and other cytoskeletal functions.<sup>3</sup> Thus, temporal and spatial regulation of microtubule assembly is critical for the correct assembly of the mitotic apparatus and of the cytoplasmic microtubule array. The major building block of microtubules is tubulin, an intracellular cylindrical filamentous structure that is present in almost all eukaryotic cells.

Except in the simplest eukaryotes, tubulin exists in all cells as a 100 kDa protein, a heterodimer of two similar but not identical polypeptides (approximately 55 kDa each) designated  $\alpha$  and  $\beta$ , that assemble into microtubules. Within either family of  $\alpha/\beta$  tubulin heterodimer, individual subunits diverge from each other (both within and across species) at less than 10% of the amino acid positions.<sup>4</sup> The most extreme diversity is localized to the carboxy-terminal 15 residues.

Both  $\alpha$ - and  $\beta$ -tubulins consist of various isotypes. In addition, both undergo post-translational modifications, including acetylation, phosphorylation, dephosphorylation, polyglutamylation, and polyglycylation.<sup>5</sup> For  $\beta$ -tubulin, six evolutionarily conserved isotypes were identified (designated  $\beta_I$ -  $\beta_{VI}$ ). Their utilization in the same cell type of different species is nearly absolutely conserved, with the exception of the hematopoietic  $\beta$ -tubulin, which is highly divergent in sequence and is not conserved between species. Research has been centered around the hypothesis that these  $\beta$ -tubulin isotypes contribute unique functional properties, since the different isotypes of tubulin differ from each other in their ability to polymerize into microtubules.<sup>6</sup> The most complex pattern of isotype distribution in tissues is seen in the vertebrate  $\beta$ -tubulins.<sup>7</sup>

In mammals and birds,  $\beta_I$  is constitutive and found in most tissues.  $\beta_{II}$  is found in many tissues, but largely in the brain and its synthesis increases in regeneration and development of neurons.  $\beta_{III}$  is found in the brain and in dorsal root ganglia and it appears to be localized to neurons, where its expression seems to increase during axonal outgrowth.  $\beta_{III}$  is also found in Sertoli cells of the testis, and in certain tumors of non-neural origin, such as lymphoma, squamous cell carcinoma, and malignant melanoma, but does not appear to be expressed in those tissues before transformation.  $\beta_{IV}$  is somewhat complex: in mammals, it exists as two subtypes, differing from each other at 10 positions.

$\beta_{IVa}$  is brain specific, whereas  $\beta_{IVb}$  is ubiquitous, and both appear to be constitutive. In chickens, there is only one form of  $\beta_{IV}$ , which is expressed at low levels in many tissues, but is the major  $\beta$  isotype in the testis.  $\beta_V$  in chickens is apparently ubiquitous outside of the brain and is also expressed in a variety of cultured mammalian cells.  $\beta_{VI}$  is apparently restricted to hematopoietic tissues, being expressed in chicken erythrocytes and in mammalian platelets, spleen, bone marrow, and other blood-forming tissues.

The detection, localization, and characterization of proteins involved in microtubule function are fundamental to the understanding of mitosis, meiosis, organellar and flagellar movement, intracellular transport, and cytoskeletal functions. Antibodies reacting specifically with  $\alpha$ - and  $\beta$ -tubulin isotypes serve as an essential tool for the detection of the presence and functional significance of these molecules in various cellular settings. The D66 antibody reacted specifically with a subset of  $\beta$ -tubulin isoforms and interferes with motility by affecting the flagellar beat frequency of sea urchin spermatozoa.<sup>1</sup>

## Reagent

Supplied at ~1 mg/mL in a solution of 0.01 M phosphate buffered saline, pH 7.4, containing 1% bovine serum albumin (BSA) and 15 mM sodium azide.

## Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

## Storage/Stability

For continuous use, store at 2-8 °C for up to one month. For prolonged storage, freeze in working aliquots at -20 °C. Repeated freezing and thawing, or storage in "frost-free" freezers, is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

## Product Profile

### Immunoblotting:

A minimum working dilution of 0.5-1  $\mu$ g/mL is determined using a whole extract of cultured rat adrenal pheochromocytoma (PC-12) cells.

**Note:** In order to obtain best results in different techniques and preparations we recommend determining optimal working dilutions by titration test.

## References

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6. Banerjee, A., et al., *J. Biol. Chem.*, 265, 1794 (1990).
7. Luduena, R.F., *Mol. Biol. Cell*, 4, 445 (1993).

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T0198dat Rev 07/22

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