



**For Research Use Only.**  
**Not for use in diagnostic procedures.**

### SPECIFICITY

Tubulin, a heterodimeric protein composed of  $\alpha$  and  $\beta$  monomers, is the major building block of microtubules.<sup>1</sup> Microtubules are responsible for such vital cell functions as cell division, transport of membrane vesicles within the cytoplasm and the maintenance of cell shape. Endotoxin binding by microtubules in monocytes and macrophages regulates inflammatory events such as cytokine production.<sup>2</sup>

These proteins are GTPases that belong to the tubulin superfamily, sharing approximately 60% amino acid identity within the monomeric species.<sup>3</sup> At least six different families have been elucidated to date. The microtubule is composed of  $\alpha$  and  $\beta$  monomers while the  $\gamma$  tubulin contributes to the nucleation of the microtubule assembly. Eukaryote cells contain  $\alpha$ ,  $\beta$ , and  $\gamma$  -tubulin, while the other tubulins are not as ubiquitous or are found only in specific organisms.<sup>3</sup>

The structure of tubulin, determined by electron crystallography, indicates that there are three functional domains within each monomer. One domain binds nucleotides, a second area binds to drugs (for example, taxol) while the third region binds other proteins.<sup>1,4</sup> Compounds that bind to the second functional domain of tubulin (for example, drugs) prevent the cell from dividing making this domain a desired target of cancer chemotherapeutic drugs.<sup>5</sup>

Anti-tubulin monoclonal antibody has been demonstrated to be useful as a viability marker in flow cytometric analysis of cell populations containing apoptotic or necrotic cells.<sup>6-10</sup> The TB1A337.3 monoclonal antibody recognizes a determinant on the tubulin heterodimer that is comprised of the  $\alpha$  and  $\beta$  monomers.

### REAGENTS

IOtest Tubulin-FITC Conjugated Antibodies  
PN 6607113 - 50 tests - 20  $\mu$ L/test

**CLONE:** TB1A337.3

**ISOTYPE:** IgG1

**IMMUNOGEN:** Bovine brain tubulin protein

**HYBRIDOMA:** P3 x A/J

**SOURCE:** Ascites fluid

**PURIFICATION:** Affinity chromatography

**CONJUGATION:** FITC is conjugated at a Molar Ratio FITC/Ig: 4-7.

Excitation wavelength at 486-580 nm.

Emission wavelength at 504-541 nm.

**BUFFER:** 2 mg/mL bovine serum albumin in phosphate-buffered saline containing 0.1% sodium azide.

### STATEMENT OF WARNINGS

1. This reagent contains 0.1% sodium azide. Sodium azide under acid conditions yields hydrazoic acid, an extremely toxic compound. Azide compounds should be flushed with running water while being discarded. These precautions are recommended to avoid deposits in metal piping in which explosive conditions can develop. If skin or eye contact occurs, wash excessively with water.
2. Specimens, samples and all material coming in contact with them should be handled as if capable of transmitting infection and disposed of with proper precautions.
3. Never pipet by mouth and avoid contact of samples with skin and mucous membranes.
4. Do not use antibody beyond the expiration date on the label.
5. Do not expose reagents to strong light during storage or incubation.
6. Use Good Laboratory Practices (GLP) when handling reagent.
7. Harmful if swallowed.
8. After contact with skin, wash immediately with plenty of water.

### STORAGE CONDITIONS AND STABILITY

This reagent is stable up to the expiration date when stored at 2-8°C. Do not freeze. Minimize exposure to light.

### REAGENT PREPARATION

No reconstitution is necessary. This monoclonal antibody may be used directly from the vial. Bring reagent to 18-25°C prior to use.

### PROCEDURE

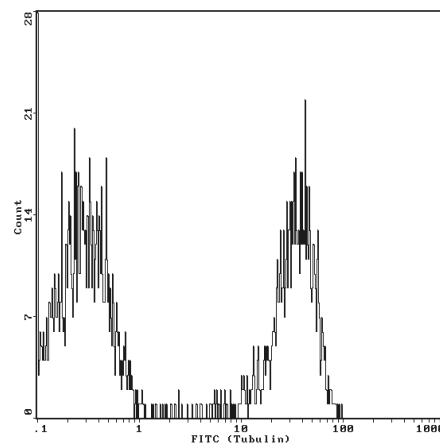
This reagent is designed for flow cytometry. Assay volume: 20  $\mu$ L per  $5 \times 10^5$  cells in one test, or per 100  $\mu$ L whole blood. A wash is required to yield optimal results.

### EXAMPLE DATA

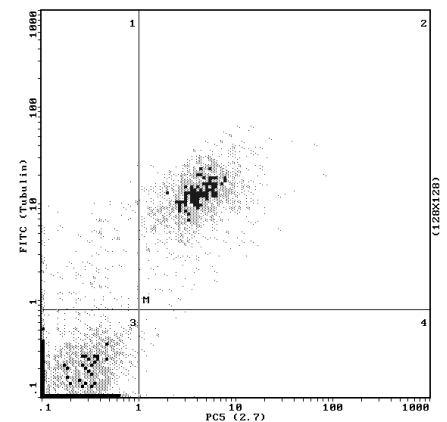
The histograms shown are representative of captothecin-induced Jurket cells stained with Tubulin-FITC monoclonal antibody (PN 6607113) and APO2.7-PC5 (PN IM2660).

Acquisition with a COULTER® EPICS® XL-MCL™ flow cytometer.

**Figure 1:**  
**Count vs. Fluorescence Intensity**



**Figure 2:**  
**Tubulin-FITC vs. APO2.7-PC5**



### SELECTED RESEARCH REFERENCES

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