

**Tubulite™ Green \*Cell-Permeable\***

 Catalog number: 23717  
 Unit size: 100 Slides

Component	Storage	Amount (Cat No. 23717)
Tubulite™ Green	Freeze (< -15 °C), Minimize light exposure	100 Slides

**OVERVIEW**

Tubulite™ Green is a robust tool for fluorescent visualization of tubulin structures in live cells. This cell-permeable probe enables real-time imaging of tubulin dynamics, bypassing the need for cell fixation and allowing continuous assessment of tubulin polymerization and organization. The probe's green fluorescence and high cellular permeability facilitate its use in multiplexed imaging applications, including co-staining with RFP and nuclear dyes such as DAPI. Upon cellular entry, Tubulite™ Green is hydrolyzed by intracellular esterases, removing its lipophilic blocking group to produce a charged, membrane-impermeant form that is well-retained within cells. Tubulite™ Green is not generally recommended for studies of dynamic cellular processes, as it may interfere with key cellular functions, including tubulin activity and cell division. Additionally, it is unsuitable for fixed-cell imaging, as fluorescence is lost during cell fixation.

**AT A GLANCE**
**Protocol Summary**

1. Prepare cells with test compounds at a density of  $5 \times 10^5$  to  $1 \times 10^6$  cells/mL
2. Prepare and add Tubulite™ Green working solution to cells
3. Incubate at 37°C for 60 minutes.
4. Use a fluorescence microscope with a FITC filter set to measure fluorescence intensity.

**Note:** This protocol is intended as a general guide and should be adjusted to meet your specific requirements.

**KEY PARAMETERS**
**Fluorescence microscope**

Emission	FITC filter set
Excitation	FITC filter set
Recommended plate	Black wall/clear bottom

**CELL PREPARATION**
**Prepare Cells**

1. For each sample, prepare cells in 1 mL of warm medium or buffer at a density of  $5 \times 10^5$  to  $1 \times 10^6$  cells/mL.

**Note:** Each cell line should be individually assessed to determine its optimal cell density.

**PREPARATION OF STOCK SOLUTIONS**

*Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles*

**Tubulite™ Green Stock Solution**

1. Add 25 µL of DMSO (not provided) to the Tubulite™ Green vial and mix thoroughly.

**Note:** Aliquot and store any unused Tubulite™ Green stock solution at -20°C. Avoid multiple freeze-thaw cycles.

**PREPARATION OF WORKING SOLUTION**
**Tubulite™ Green Working Solution (1X)**

1. To prepare a 1X Tubulite™ Green working solution, add 2.5 µL of Tubulite™ Green stock solution and 1 mM ReadiUse™ Probenecid (AAT Cat# 20061, not provided) to 1 mL of HHBS [Hanks' Buffer with 20 mM Heps] (AAT Cat# 20011, not provided) or your preferred buffer. Mix thoroughly.

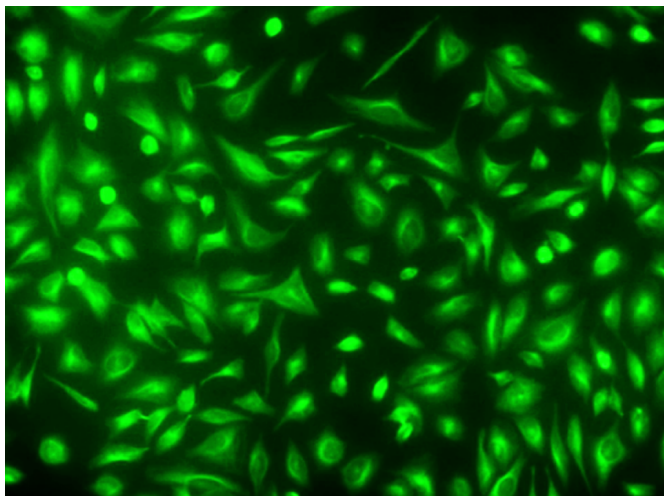
**Note:** For best results, we recommend preparing a fresh Tubulite™ Green working solution each time you use it. The working solution remains stable for a few hours.

**Note:** The calculations above assume that 100 µL of working solution will be used for each slide or sample. Please adjust the concentrations of Tubulite™ Green and ReadiUse™ probenecid to match your specific staining and cell conditions.

**SAMPLE EXPERIMENTAL PROTOCOL**

1. Prepare cell samples as needed.
2. Remove the cell growth medium, then wash the cells with PBS (not included) or another buffer of your choice.
3. Add 100 µL of Tubulite™ Green working solution to the cells, then place them in a 37°C incubator for 60 minutes.  
  
**Note:** The incubation time will vary based on the specific cell type and cell concentration. Be sure to adjust the incubation time to best suit each experiment.
4. Remove the working solution and wash the cells twice with PBS or a preferred buffer.
5. Cover the cells with HHBS (or a buffer of your choice) containing 1 mM probenecid. Then, use a fluorescence microscope equipped with a FITC filter set to monitor the fluorescence intensity.

## EXAMPLE DATA ANALYSIS AND FIGURES



**Figure 1.** Imaging of tubulin in live HeLa cells: HeLa cells were labeled for 60 minutes at 37°C with Tubulite™ Green (#23717). Fluorescent images were captured using a fluorescence microscope with a FITC filter set.

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