# Tubulite™ Deep Red *Cell-Permeable*

Catalog number: 23719
Unit size: 100 Slides

| Component | Storage | Amount (Cat No. 23719) |
|---|---|---|
| Tubulite™ Deep Red | Freeze (< -15 °C), Minimize light exposure | 100 Slides |

## OVERVIEW

Tubulite™ Deep Red 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 deep red fluorescence and high cellular permeability facilitate its use in multiplexed imaging applications, including co-staining with GFP and nuclear dyes such as DAPI. Upon cellular entry, Tubulite™ Deep Red is hydrolyzed by intracellular esterases, removing its lipophilic blocking group to produce a charged, membrane-impermeant form that is well-retained within cells. Tubulite™ Deep Red 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 Tublite™ Deep Red working solution to cells
3. Incubate at 37°C for 60 minutes.
4. Use a fluorescence microscope with a Cy5 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 | Cy5 filter set |
| Excitation | Cy5 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™ Deep Red Stock Solution (100X)**

1. Add 100 µL of DMSO (not provided) to the Tublite™ Deep Red vial and mix thoroughly.

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

## PREPARATION OF WORKING SOLUTION

**Tubulite™ Deep Red Working Solution (1X)**

1. To prepare a 1X Tubulite™ Deep Red working solution, add 10 µL of Tublite™ Deep Red stock solution and 1 mM ReadiUse™ Probenecid (AAT Cat# 20061, not provided) to 1 mL of HHBS [Hanks' Buffer with 20 mM Hepes] (AAT Cat# 20011, not provided) or your preferred buffer. Mix thoroughly.

   **Note:** For best results, we recommend preparing a fresh Tublite™ Deep Red 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 Tublite™ Deep Red 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 Tublite™ Deep Red 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 Cy5 filter set to monitor the fluorescence intensity.

## EXAMPLE DATA ANALYSIS AND FIGURES

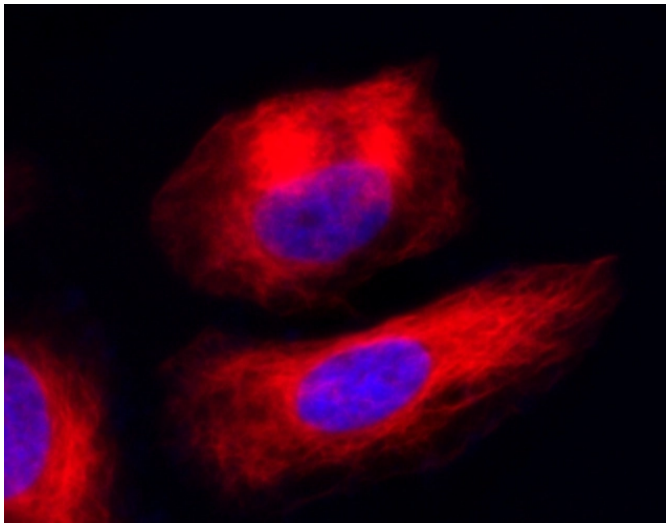

**Figure 1.** Imaging of Tubulin in Live HeLa Cells: HeLa cells were co-labeled with Tubulite™ Red and DAPI (Cat# 17507) and incubated for 60 minutes at 37°C in a 5% $CO_2$ atmosphere. Fluorescence imaging was performed using a fluorescence microscope equipped with a Cy5 filter set.

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