Cell Navigator® Live Cell Tubulin Staining Kit

Catalog number: 23170, 23171
Unit size: 100 Slides, 300 Slides

Component	Storage	Amount (Cat No. 23170)	Amount (Cat No. 23171)
Component A: Tubulite™ Deep Red	Freeze (< -15 °C), Minimize light exposure	1 vial	3 vials
Component B: Assay Buffer	Freeze (< -15 °C)	1 bottle (20 mL)	1 bottle (60 mL)
Component C: 25 mM ReadiUse™ probenecid (10X)	Freeze (< -15 °C), Minimize light exposure	1 bottle (10 mL)	2 bottles (10 mL/bottle)
Component D: DMSO	Freeze (< -15 °C)	1 vial (100 µL)	1 vial (100 µL)

OVERVIEW

The Cell Navigator® Live Cell Tubulin Staining Kit provides a robust method for the fluorescent visualization of tubulins in live cells using Tubulite™ Deep Red. This cell-permeable probe enables live-cell imaging of tubulin dynamics without requiring fixation, facilitating real-time monitoring of tubulin polymerization. The deep red fluorescence of Tubulite™ Deep Red and efficient cellular permeability allows for multiplexing with other fluorescent labels, including GFP and nuclear stains such as DAPI. Once Tubulite™ Deep Red traverses the plasma membrane, cellular esterases hydrolyze the lipophilic blocking group, yielding a charged, membrane-impermeant product that remains well-retained within the cell, enabling sustained and stable imaging of intracellular tubulin structures.

AT A GLANCE

Protocol Summary

1. Prepare cells with test compounds at a density of 5×10^5 to 1×10^6 cells/mL
2. Prepare and add Tubulite Deep™ Red working solution to cells
3. Incubate at 37 °C for 30 to 60 minutes
4. Read fluorescence intensity with Cy5 filter set

Important Note

Thaw one of each kit component at room temperature before starting the experiment.

Note: This protocol only provides a guideline, and should be modified according to your specific needs.

Note: Tubulite™ Deep Red does not stain formaldehyde-fixed cells. Cells can not be fixed after staining with Tubulite™ Deep Red as fixation alters the structure of microtubules.

KEY PARAMETERS

Fluorescence microscope

Emission	Cy5 filter set
Excitation	Cy5 filter set
Recommended plate	Black wall/clear bottom

CELL PREPARATION

For guidelines on cell sample preparation, please visit:

https://www.aatbio.com/resources/guides/cell-sample-preparation.html

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 (500X)

1. Add 25 µL DMSO (Component D) into the vial of Tubulite™ Deep Red (Component A), and mix well.

   Note: Aliquot and store the unused Tubulite™ Deep Red stock solution at -20 °C. Avoid repeated freeze/thaw cycles.

PREPARATION OF WORKING SOLUTION

Tubulite™ Deep Red working solution (1X)

1. Add 2.5 µL of Tubulite™ Deep Red stock solution stock solution and 100 µL 25 mM ReadiUse™ probenecid (Component D) into 1 mL of Assay Buffer (Component B) or buffer of your choice, and mix well.

   Note: We recommend making Tubulite™ Deep Red working solution fresh for every use. The working solution is stable for several hours.

SAMPLE EXPERIMENTAL PROTOCOL

1. Prepare cell samples as per need.
2. Remove the cell growth medium and wash cells with PBS (Not provided) or any other buffer of your choice. (Optional).
3. Add 100 µL Tubulite™ Deep Red working solution and incubate them at 37 °C incubator for 30 to 60 minutes.

   Note: The appropriate incubation time depends on the individual cell type and cell concentration used. Optimize the incubation time for each experiment.
4. Remove the working solution and wash cells twice with PBS or any other buffer of your choice with 2.5 mM probenecid (diluted from Component C).
5. Cover the cells with an Assay Buffer containing 2.5 mM probenecid (prepared by diluting Component C), and then monitor fluorescence intensity using a fluorescence microscope with a Cy5 filter set.

EXAMPLE DATA ANALYSIS AND FIGURES

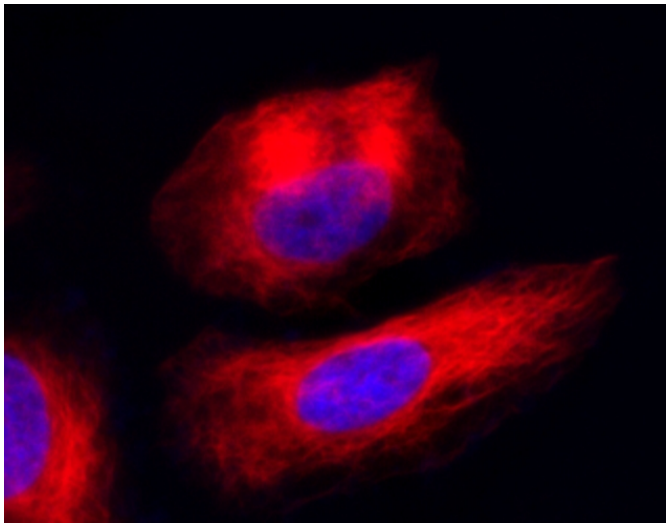

Figure 1. Imaging of Tubulins in Live HeLa Cells: HeLa cells were co-labeled with Tubulite™ Deep Red and DAPI (Cat# 17507) and incubated for 60 minutes at 37°C in a 5% CO_2 environment. Fluorescent images were captured using a fluorescence microscope with a Cy5 filter set.

DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email info@aatbio.com if you have any questions.