Cell Navigator™ Live Cell Tubulin Staining Kit

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

Component	Storage	Amount	
		Cat No. 23170	Cat No. 23171
Component A: Tubulite™ Red	Freeze (<-15 °C), Minimize light exposure	1 vial	1 vial
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

Cell Navigator™ Live Cell Tubulin Staining Kit provides a robust method to fluorescently image tubulins in live cells with Tubulite™ Red. The probe is permeant to live cells, thus does not require cells to be fixated for imaging tubulins. It can be conveniently used for tracking tubulin polymerization process in live cells. Its red spectral wavelengths and good cell permeability make this probe readily to use with other colors such as GFP expressed cells or nuclear dyes such as DAPI. The neutral Tubulite™ Red readily passes through the plasma membranes of live cells. Once inside the cells, the lipophilic blocking group is cleaved by esterases, resulting into a negatively charged product, which is well retained inside the cells.

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™ Red working solution to cells
3. Incubate at 37°C for 30 to 60 minutes
4. Read fluorescence intensity with Cy5 filter set

Important 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.

KEY PARAMETERS

Instrument:	Fluorescence microscope
Excitation:	Cy5 filter set
Emission:	Cy5 filter set
Recommended plate:	Black wall/clear bottom

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™ Red stock solution (500X):
Add 25 µL (**Cat#23170**) or 75 µL (**Cat#23171**) DMSO (Component D) into the vial of Tubulite™ Red (Component A), and mix well.

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

PREPARATION OF WORKING SOLUTION

Tubulite™ Red working solution (1X):
Add 2.5 µL of Tubulite™ 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™ Red working solution fresh for every use. The working solution is stable for several hours.

PREPARATION OF CELL SAMPLES

For guidelines on cell sample preparation, please visit
https://www.aatbio.com/resources/guides/cell-sample-preparation.html

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™ 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 cells with Assay Buffer with 2.5 mM probenecid (diluted from Component C) and monitor the fluorescence intensity with fluorescence microscope using Cy5 filter set.

EXAMPLE DATA ANALYSIS AND FIGURES

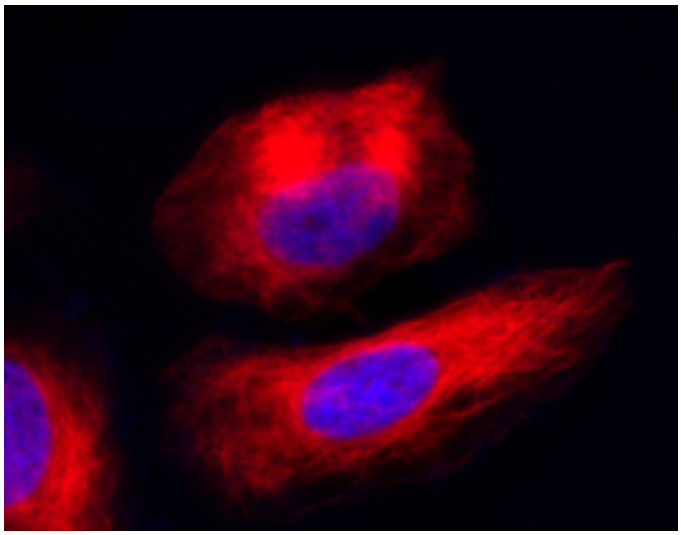

Figure 1. Imaging of tubulins in live HeLa cells. HeLa cells were co-labelled with Tubulite™ Red and DAPI (Cat# 17507) for 60 minutes in a 37 °C, 5% CO_2 incubator.

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The fluorescence image was taken with a fluorescence microscope (Cy5 filter set).

DISCLAIMER