ab324125 – Live Cell Tubulin Staining Kit (Fluorimetric)

A robust method for the fluorescent visualization of tubulins in live cells using Tubulite™ Deep Red.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab324125

Storage and Stability: Store kit at -20°C in the dark immediately upon receipt. Refer to list of materials supplied for storage conditions of individual components.

Materials Supplied

Item	Quantity	Storage Condition
Tubulite™ Deep Red	1 vial	-20°C (Store in the dark)
Assay Buffer	1 bottle (20 mL)	-20°C
25 mM ReadiUse™ probenecid (10X)	1 bottle (10 mL)	-20°C (Store in the dark)
DMSO	1 vial (100 µL)	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Fluorescence microscope – Cy5 filter set
- Black wall/ clear bottom plates
- PBS
- 37 °C incubator

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: 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.*

***Note**: Cells cannot be fixed after staining with Tubulite™ Deep Red as fixation alters the structure of microtubules.*

Preparation of Stock Solution

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 into the vial of Tubulite™ Deep Red, 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 into 1 mL of Assay Buffer 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.*

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 25 mM ReadiUse™ probenecid (10X)).
5. Cover the cells with an Assay Buffer containing 2.5 mM probenecid (prepared by diluting 25 mM ReadiUse™ probenecid (10X)), and then monitor fluorescence intensity using a fluorescence microscope with a Cy5 filter set.

For technical support contact information, visit: www.abcam.com/contactus

Version 1 | 2025-05-06