

Tubulin Tracker™ Green and Tubulin Tracker™ Deep Red

Cellular staining with Tubulin Tracker™ reagents

Catalog Numbers T34075, T34076, T34077, T34078, and T34079

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

The Invitrogen™ Tubulin Tracker™ reagents provide fluorescent staining of polymerized tubulin in live cells. They are designed to readily permeate live cells, thus providing more uniform labeling and better selectivity compared to other methods of detection.

Tubulin Tracker™ Green (Oregon Green™ 488 Taxol, bis -acetate) is an uncharged compound that easily passes through the plasma membrane of live cells. Once inside the cell, the lipophilic blocking group is cleaved by nonspecific esterases, resulting in a charged form that is retained within the cells better than the uncharged form.

Tubulin Tracker™ Deep Red is a conjugate of Docetaxel and a very bright and photostable deep red fluorophore that easily enters the cells and specifically labeled polymerized tubulin.

Taxol™/Paclitaxel and Taxotere™/Docetaxel belong to a drug family that promotes/preserve tubulin polymerization and can interfere with the cellular functions where tubulin de-polymerization is needed, such as cellular division.

In Figure 1, the cellular viability of HeLa cells is detected with Tubulin Tracker™ Green and Tubulin Tracker™ Deep Red at 1X concentration compared to vehicular control (DMSO solution). The data indicate that in HeLa cells, the cellular viability is not compromised in 24 hours.

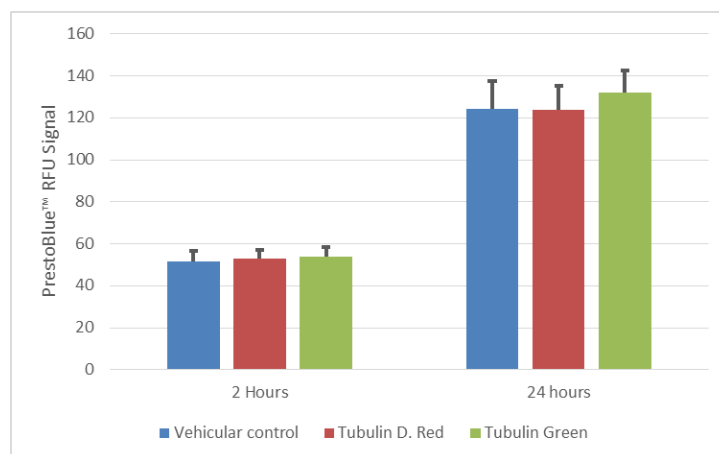


Figure 1 HeLa cellular viability

HeLa cells incubated for 2 hours with 1µM of Tubulin Tracker™ Deep Red (Cat. No. T34076) and Tubulin Tracker™ Green (Cat. No. T34075) show minimal cytotoxicity compared with control cells incubated with the control (DMSO). Cytotoxicity was measured using PrestoBlue™ Cell Viability Reagent (Cat. No. A13262) on a Varioskan™ LUX (Cat. No. VLBLATD0) multimode microplate reader. (Error bars: standard error of the mean, n = 4.)

Spectral properties

- Tubulin Tracker™ Green utilizes Oregon Green™ 488 dye which absorbs and emits optimally at 494 nm and 522 nm, respectively (see Figure 2). Fluorescence is visualized with a standard FITC or GFP filter.
- Tubulin Tracker™ Deep Red utilizes a photostable, bright deep red fluorophore, which absorbs and emits optimally at 652 nm and 669 nm, respectively (see Figure 2). Fluorescence is visualized with a standard Cy5 filter.

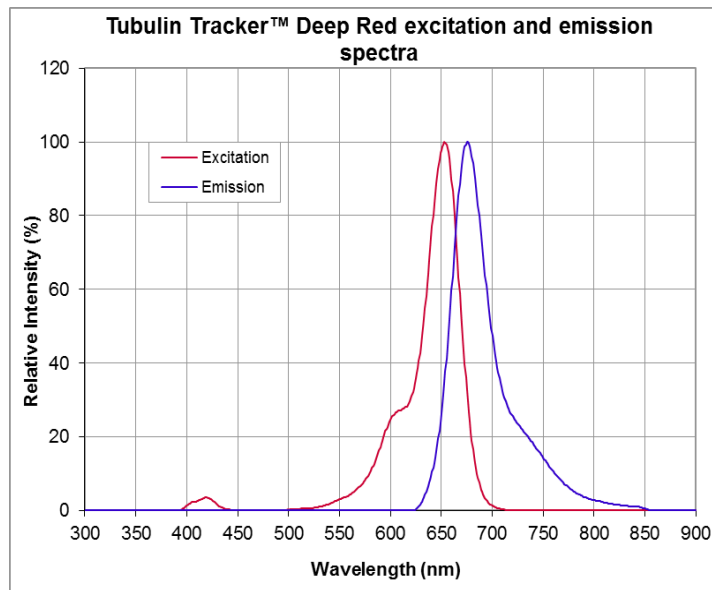
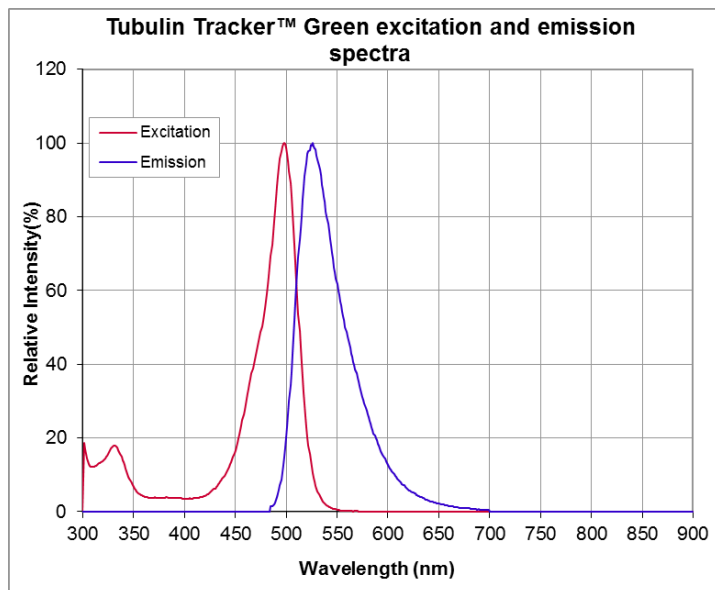


Figure 2 Tubulin Tracker™ reagents excitation and emission spectra

Contents and storage

The Tubulin Tracker™ reagents provide sufficient assays for staining 60 (T34077, T34078, and T34079) and 300 slides (T34075, T34076) when using 1 mL of 1X staining solution per slide and performing the incubation in 6-well plates.

More slides/assays can be processed by using less volume per slide/assay. The following protocol can be easily adapted to other formats, such as 96-well plates or other well plate formats.

Table 1 Tubulin Tracker™ Green

Contents	Cat. No. T34078 (60 slides)	Cat. No. T34075 (300 slides)	Storage
Tubulin Tracker™ Green (Oregon Green™ 488 Taxol, bis - acetate)	1 vial (20 µg/vial; lyophilized)	1 vial (100 µg/vial; lyophilized)	Store at -5°C to -20°C after receiving in a freezer in dark, desiccated chamber. Do not freeze/thaw. When stored as instructed, these reagents can be used for at least 6 months after receiving.
Pluronic F-127 20% solution in DMSO	1 vial (200 µL/vial)	1 vial (1 mL/vial)	
Probenecid	1 vial (77 mg/vial; lyophilized)	—	

Table 2 Tubulin Tracker™ Deep Red

Contents	Cat. No. T34077 (60 slides)	Cat. No. T34076 (300 slides)	Storage
Tubulin Tracker™ Deep Red	1 vial	5 vials	Store at -5°C to -20°C after receiving in a freezer in dark, desiccated chamber. When stored as instructed, these reagents can be used for at least 6 months after receiving.
Probenecid	1 vial (77 mg/vial; lyophilized)	5 vials (77 mg/vial; lyophilized)	

Table 3 Tubulin Tracker™ Variety Pack (Cat. No. T34079)

Contents	Amount	Storage
Tubulin Tracker™ Green (Cat. No. T34078)	1 kit	Store at -5°C to -20°C after receiving in a freezer in dark, desiccated chamber.
Tubulin Tracker™ Deep Red (Cat. No. T34077)	1 kit	Do not freeze/thaw. When stored as instructed, these reagents can be used for at least 6 months after receiving.

Materials required not supplied

Unless otherwise indicated, all materials are available through thermofisher.com.

- Live specimen, such as cultured or primary cells, 3D cell cultures, spheroids, or organoids
- Anhydrous DMSO (Cat. No. D12345)
- Live cell-compatible buffer:
 - Live Cell Imaging Solution (Cat. No. A14291DJ)
 - HBSS with calcium and magnesium (Cat. No. 24020117)
 - FluoroBrite™ DMEM (Cat. No. A1896701)
- (Optional) Probenecid, Water Soluble (Cat. No. P36400)
- (Optional) Pluronic™ F-127-20% Solution in DMSO (Cat. No. P300MP)
- (Optional) NucBlue™ Live ReadyProbes™ Reagent (Cat. No. R37605)
- (Optional) BackDrop™ Background Suppressor (Cat. No. B10511)

Procedural guidelines

- Recommendations for experimental protocols should be used as a starting point, and optimal labeling conditions for each cell type should be determined empirically.
- The Pluronic F-127 solution enhances the loading of Tubulin Tracker™ Green in live cells, but does not seem to enhance the loading of Tubulin Tracker™ Deep Red. For best results, vortex well solutions that contain Pluronic F-127 before use.
- Probenecid prevents efflux of fluorescent reagents in many live cell types. To minimize off-target effects on other cellular functions, we recommend to use Probenecid at 1X concentration while loading and imaging with Tubulin Tracker™ Green and Tubulin Tracker™ Deep Red. Do not incubate in Probenecid for more than a few hours.

Before you begin

- Make a 4000X stock solution of Tubulin Tracker™ Green by dissolving the content of the vial in 15 µL (Cat. No. T34078) or 75 µL (Cat. No. T34075) of anhydrous DMSO.

This stock solution is stable for at least three months when stored at ≤-20°C.

- *For Tubulin Tracker™ Green only:* add an equal volume of Pluronic F-127 (20% solution in DMSO) to the Tubulin Tracker™ Green stock solution. This 2000X intermediate stock solution is stable for 14 days when stored at ≤-20°C.
- Make a 1000X stock solution of Tubulin Tracker™ Deep Red by dissolving the content of the vial in 60 µL of anhydrous DMSO. This stock solution is stable for at least three months when stored at ≤-20°C.
- Dissolve Probenecid in 1 mL of live cell compatible buffer to make a 100X stock solution. This solution is stable for 6 months when stored at ≤-20°C.

Prepare and stain the cells with Tubulin Tracker™ Green

1. Vortex well the intermediate stock solution.
2. Dilute the intermediate stock solution to 1X in live-cell compatible buffer. Lower concentrations can be used in certain cell types. Use only solution freshly made on the same day.
3. Apply a sufficient amount of the final staining solution to cover cells adhering to vessel.
4. Incubate for 30 minutes at 37°C and 5% CO₂.
(Optional) Nucleus staining reagent can be added at 1X concentration.
(Optional) 1X BackDrop™ Background Suppressor can be used to reduce background. If BackDrop™ Background Suppressor is used, proceed directly to step 6.
When staining a 3D cell culture such as spheroid or organoid, the same final staining concentration is recommended, but with a prolonged incubation time to allow complete penetration of the label.
5. Rinse the cells 3 times in a wash buffer such as Live Cell Imaging Solution at 37°C.
(Optional) Add 1X Probenecid to the wash buffer to minimize efflux of probe during rinse and imaging steps. Do not leave cells in Probenecid for more than 2 hours as it can interfere with some cellular functions.
6. Image and analyze cells in buffer. Samples should be viewed within few hours after staining, as staining intensity will diminish with time.

Prepare and stain cells with Tubulin Tracker™ Deep Red

1. Dilute the stock solution to 1X in live-cell compatible buffer.

Lower concentrations can be used in certain cell types.

Use only solution freshly made on the same day.

2. Apply a sufficient amount of the final staining solution to cover cells adhering to vessel.

3. Incubate for 30 minutes at 37°C and 5% CO₂.

(Optional) Nucleus staining reagent can be added at 1X concentration.

(Optional) 1X BackDrop™ Background Suppressor can be used to reduce background. If BackDrop™ Background Suppressor is used, proceed directly to step 5.

When staining a 3D cell culture such as spheroid or organoid, the same final staining concentration is

recommended, but with a prolonged incubation time to allow complete penetration of the label.

4. Rinse the cells 3 times in a wash buffer such as Live Cell Imaging Solution at 37°C.

(Optional) Add 1X Probenecid to the wash buffer to minimize efflux of probe during rinse and imaging steps. Do not leave cells in Probenecid for more than 2 hours as it can interfere with some cellular functions.

5. Image and analyze cells in buffer.

Samples should be viewed within few hours after staining, as staining intensity will diminish with time.

Limited product warranty

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