

Data Sheet

BioTracker™ 497 Green Actin Live Cell Probe

Live Cell Probe

SCT215**Pack Size: 5 vials x 30 nmol****Store at -20 °C****FOR RESEARCH USE ONLY****Not for use in diagnostic procedures. Not for human or animal consumption.**

Background

Actin, a key protein in contractile filaments of muscle cells, is also essential in diverse and critical cellular processes. These include cell motility, cytokinesis, organelle movement, phagocytosis, cell division, cell signaling, and maintenance of cell shape and junctions. Most reagents for the fluorescent detection of actin are not intended for use as live cell probes due to permeability barriers and toxicity to living cells.

The BioTracker™ 497 Green Actin Live Cell Probe is a fluorescent probe that specifically binds to actin filaments (F-actin) and enables visualization with green fluorescence. It is applicable to both living and fixed cell preparations. Because SCT215 is an organic fluorescent small molecule with high membrane permeability, it is possible to visualize intracellular actin filaments by adding to the cell culture medium or extracellular fluid without a rinsing step. This probe is thought to bind to F-actin through the common binding site for phalloidin and jasplakinolide.

Compared with traditional methods for fluorescent actin staining, BioTracker™ 497 Green Actin Live Cell Probe (SCT215):

- Performs consistently under a variety of incubation times and concentrations
- Live cell protocol is very easy and convenient; fewer steps, reagents and time are required compared with a traditional immunocytochemistry/immunofluorescence protocol for actin detection

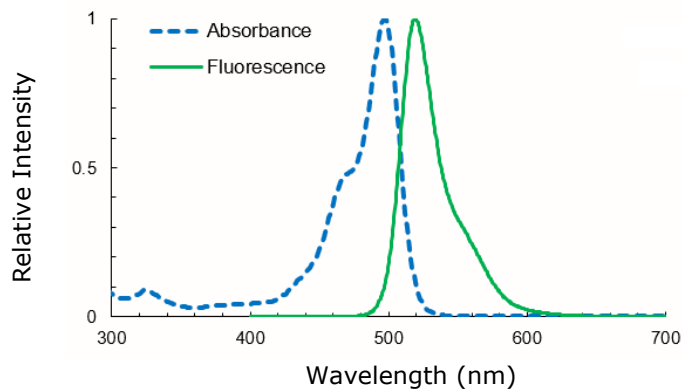
Source

BioTracker™ 497 Green Actin Live Cell Probe does not contain genetically modified organisms.

Spectral Properties

Excitation peak: 497 nm

Emission peak: 520 nm



Presentation

BioTracker™ 497 Green Actin Live Cell Probe (SCT215) is an orange solid.

Storage and Handling

BioTracker™ 497 Green Actin Live Cell Probe is shipped as a dried solid in a nitrogen gas-filled vial. Store at -20°C , desiccated and protected from light. After dissolving in DMSO, store as small aliquots at $\leq -20^{\circ}\text{C}$. Avoid freeze-thaw cycles and use aliquots as soon as possible after thawing.

Representative Data

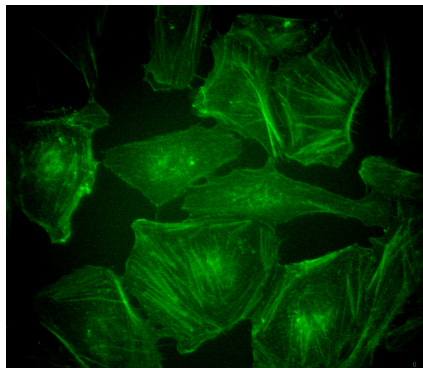


Figure 1. Fixed HeLa cells were incubated for 30 minutes in 100 nM BioTracker™ 497 Green Actin Live Cell Probe.

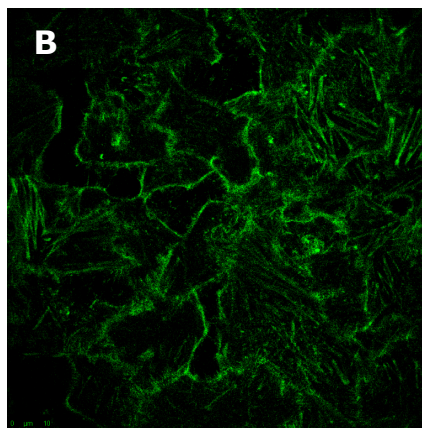
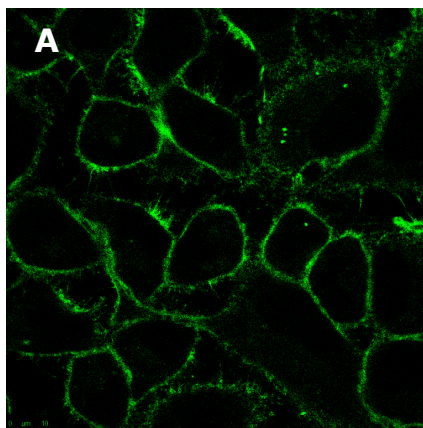


Figure 2. Live cell fluorescence detection of actin using 100 nM BioTracker™ 497 Green Actin Live Cell Probe HeLa cells. **(A)** External structure. **(B)** Internal cytoskeleton.

Protocols

Prepare BioTracker™ 497 Green Actin Live Cell Probe

Before opening the cap, warm the vial to room temperature. Then, use a microcentrifuge to spin down any solid that may be adhering to the cap or vial walls.

Add 30 μ L of DMSO to one vial to prepare 1 mM solution. Dissolve the solid entirely by pipetting up and down ~5 times. The solution will become a pale-yellow liquid.

Example protocol for staining live cells

1. Culture cells on a glass-bottomed or other optically compatible cultureware.
2. Add BioTracker™ 497 Green Actin Live Cell Probe solution to the culture medium at the final concentration of 10-100 nM*. To add 100 nM probe, prepare 10 μ M solution by diluting the 1 mM solution with PBS, then add the 1/100 volume of the 10 μ M solution to the culture medium. The final concentration of DMSO should be $\leq 0.1\%$. Gently mix the medium and incubate the dish in a CO₂ incubator for >10 minutes.
3. Observe the cells using a fluorescence microscope. It is not recommended to rinse the cells with fresh medium before imaging. Use of microscope stage top incubator may enhance results but is not a requirement.

Example protocol for staining fixed cells

1. Culture cells on a glass-bottomed or other optically compatible cultureware.
2. Fix cells by adding PBS(+) supplemented with 4% paraformaldehyde and incubating at 37 °C for 15 minutes.
3. Remove the fixation buffer from the cell culture dish and add 100 nM BioTracker™ 497 Green Actin Probe diluted in PBS.
4. Observe the cells by fluorescence microscopy.

Notes on Fluorescence Imaging

BioTracker™ 497 Green Actin Live Cell Probe fluorescence can be observed with a commonly available blue excitation and green fluorescence filter set for GFP/FITC. For live cell imaging, use lower concentrations of the probe, reduce the excitation light intensity, and use high-sensitivity cameras to reduce exposure time. Consider the use of antifade reagent for time-lapse or continuous imaging.

For the observation of the detailed actin structure, users may consider the use of a confocal microscope, a total internal reflection (TIRF) microscope, a structured illumination microscope (SIM), or super resolution radial fluctuation (SRRF). Stimulated emission depletion (STED) microscopy is also applicable. Use of 488 nm excitation laser with 592 nm depletion laser is appropriate.

Adjust the image intensity offset to reduce the fluorescence background signal derived from the unbound probe in the medium. Exchanging the medium to reduce the background is not recommended, because the probe concentration in the medium and the concentration of actin-bound probes form an equilibrium.

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