Technical Bulletin

BioTracker™ rRNA Live Cell Probe

Live cell imaging probe for ribosomal RNA and fluorescent detection of cell nucleoli

SCT089

Background

The BioTracker™ rRNA live cell probe is a styryl quinolinium dye that selectively stains ribosomal RNA (rRNA) in mammalian cells.[1] This probe is a fluorescent small molecule that demonstrates high affinity to rRNA, and therefore can be used as a marker for the nucleolus, where concentrations of rRNA are high. Low cytotoxicity enables the use of this probe for detection in living systems with minimal disruption to normal cellular processes.[1]

As imaging technologies have progressed over the last decade, more reagents are required to detect a variety of organoids and subcellular functions in living cells. To meet live cell imaging requirements, a fluorescent reagent should show high specificity to the subcellular or functional target along with low cytotoxicity.[2] Because the nucleolus is a dense organoid composed of chromatin, nuclear bodies and many protein components, the development of fluorescent reagent specific for live cell nucleolar detection has been even more challenging.[2, 3, 4]

The BioTracker™ rRNA live cell probe is easy to use, emits light at a longer wavelength in the visible light spectrum, and can facilitate imaging the subcellular localization of ribosomal RNA as well as serving as a marker for the nucleolus.

Source

SCT089 does not contain genetically modified organisms.

Spectral Properties

Fluorescence images were obtained by λex = 570 nm and emission at 647-667 nm.

Quality Control Testing

Purity: ≥ 95% confirmed by HPLC. Molar Mass: 298 Da confirmed by LC-MS.

Storage and Handling

Store BioTracker™ rRNA Live Cell Probe at 2-8 °C, desiccated and protected from light.

Note: Centrifuge vial briefly to collect contents at bottom of vial before opening.

Presentation

Lyophilized. Deep purple solid.

Protocols

Preparation of stock solution (for live or fixed cell staining)

1. Add 1 mL of DMSO to dissolve the powdered vial contents to a final concentration 1.0 mg/mL. Heat at 37 °C for 10 minutes. The color of the resulting solution will be deep blue.
2. Vortex to mix.
3. Verify that the solution has dissolved completely.

Preparation of working solution (for fixed cell staining)

1. Dilute stock solution in PBS to 2.5-10 µg/mL final concentration. The appearance of the solution will change to orange or orange-red color.
2. Vortex to mix.
3. Verify that the solution has dissolved completely.

Staining live cells

1. Culture the cells under standard conditions.
2. Wash cells in culture medium without FBS.
3. Incubate cells with dye stock solution diluted in culture medium with or without 10% FBS to attain a concentration of 2.5-20 µg/mL (optimal concentration will vary among different cell lines and media) under standard culture conditions, for 30 min.

   Note: It is recommended to analyze cells immediately after staining, although cells may not be affected after longer incubation with staining solution.
4. Wash cells to eliminate excess dye solution. Leave cells in PBS for imaging.
5. Analyze cells using fluorescent microscopy.

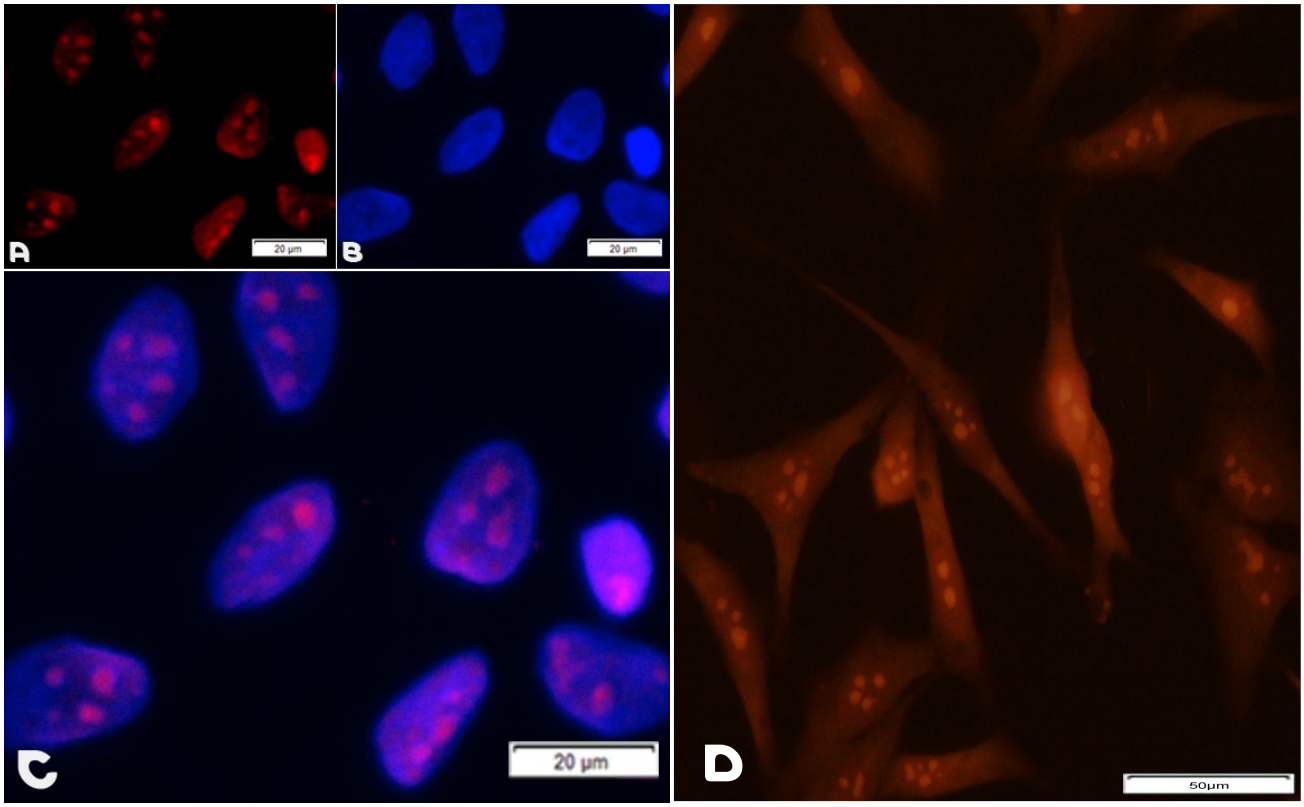
Staining fixed cells

1. Rinse 4% paraformaldehyde fixed/ 0.5% Triton X-100 permeabilized cells on glass slides with PBS.
2. Block with PBS containing 1.0% BSA for 20 minutes at ambient temperature.
3. Remove excess PBS.
4. Incubate slides with working solution as described above, for 40 minutes at ambient temperature.
5. Rinse with PBS three times for 5 min.
6. Mount the cover slide with fluorescence-suitable mounting medium.
7. Examine with fluorescence microscope using mCherry filter (600-690 nm barrier filter).

   Note: Optimal concentration must be determined by the end user.

Representative Data

Figure 1: Fluorescence microscopy images of BioTracker™ rRNA live cell probe on living (D) and fixed (A,B,C) cell samples.



A, HeLa cells were fixed and permeabilized with 4% paraformaldehyde followed by 0.5% Triton X-100. Cells were then stained with 5.0 µg/mL BioTracker™ rRNA live cell probe for 40 min, following staining (B) with 0.1 µg/mL DAPI. Cells were washed with PBS and analyzed by fluorescence microscopy, using mCherry and DAPI fluorescent filters (merged, C).

D, NIH 3T3 cells were washed with DMEM without serum, incubated with SCT089 rRNA live cell probe at a concentration of 20 µg/mL for 30 min, washed three times with PBS, and maintained in PBS for microscopic analysis. Signal was detected using fluorescent microscopy with Cy3 filter at 40x magnification. Red, SCT089 BioTracker™ rRNA Live Cell probe. Blue, DAPI.

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Technical Assistance

Visit the tech service page at SigmaAldrich.com/techservice.

Terms and Conditions of Sale

Warranty, use restrictions, and other conditions of sale may be found at SigmaAldrich.com/terms.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

References

1. Saady A *et al.* 2020. Applying styryl quinolinium fluorescent probes for imaging of ribosomal RNA in living cells. Dyes and Pigments 174(4):107986.
2. Martin RM, et al. 2007. Nucleolar marker for living cells. Histochemistry and Cell Biology 127 (3): 243-251.
3. Kim, T-K, *et al.* 2019. Physicochemical properties of nucleoli in live cells analyzed by label-free optical diffraction tomography. Cells 8(7): 699.
4. Wang K *et al.* 2014. Red emissive cross-linked chitosan and their nanoparticles for imaging the nucleoli of living cells. Carbohydrate Polymers 102: 699-707.

SCT089pis Rev01/23