BioTracker™ NTP-Transporter Molecule

Live Cell Dye

Cat. # SCT064

pack size: 1 mg

Store at -20ºC

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.
NOT FOR HUMAN OR ANIMAL CONSUMPTION.

Data Sheet

Background

Nucleoside triphosphates (NTPs) are important metabolites that are involved in numerous cellular processes. However, due to their anionic nature, NTPs do not readily permeate cell membranes. To overcome this barrier, synthetic analogues that are neutral and more readily transportable are commonly used in cell-based assays. However, these synthetic analogues have a very low efficiency of conversion to active metabolites. Hence there is a need to optimize transport of NTPs into the cells.

BioTracker™ NTP-Transporter Molecule has a receptor site, for selectively binding to the NTPs, and a cell penetrating agent. The transporter can bind to NTPs and quickly move them into cells without any apparent damage to the cell membrane or decrease in cell viability. The transported NTP is rapidly liberated from the complex and available in the nuclei for incorporation into DNA. Labeled NTPs can be used for live cell imaging, cell proliferation assays, synthetic biology studies, synthesizing artificial DNA, tracking cell division, research on active antiviral and anticancer nucleotides, among other things.

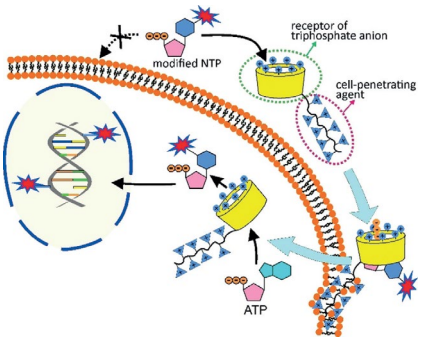

Figure 1. Hypothesized mechanism of the transport of a modified NTP by the synthetic NTP-Transporter molecule.

Storage

Store BioTracker™ ™ NTP-Transporter Molecule at -20°C, desiccate and protect from light

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

Quality Control

Purity: ≥ 98% confirmed by HNMR, LC-MS and HPLC and elemental analysis. Molar Mass: 3470.18 g/mol, which is 0.22 micro moles/mg.

Protocol

Reagent Preparation

1. Before opening the vial, spin down the solid to the bottom by a microcentrifuge or by a desktop centrifuge.
2. Warm the vial to the room temperature and add DMSO to make a 1000X stock solution of 5-10 mM (freeze aliquots at -20°C).

Protocol for Live Imaging of DNA Labeling by Confocal Microscopy

1. Seed cells in appropriate cell culture media.
2. Incubate cells at 37°C, 5% CO_2 incubator overnight.
3. Wash cells twice with tricine buffer.
4. Incubate cells at RT for 10 min with a mixture of BioTracker NTP - Transporter Molecule (10 µM) and Cy3-dUTP (10 µM).
5. After 10 min, remove staining solution and wash cells once with 400 µL 1X PBS.
6. Add 400 µL culture media to cells and place in a confocal microscope stage incubator (37°C with 5% CO_2).
7. Capture images using an appropriate filter (ex: 550 nm, em: 570 nm) (see figure 3).

Note: *Treatment time and concertation depend on cell type. Conditions for several types are given in reference 1.*

Protocol for Cell Cycle Analysis by Flow Cytometry

1. Seed 1x10^5 cells into 6-well plate and grow in cell culture media to the desired confluency.
2. Remove medium and briefly wash cells with tricine buffer.
3. Incubate cells with a mixture (0.5 mL) of BioTracker NTP-Transporter Molecule (20 µM) and Cy3-dUTP (20 µM) in the tricine buffer for 3 min (37 °C; 5% CO2).
4. Remove staining mixture and add culture medium (2 mL, 37°C), incubate (37 °C; 5% CO_2) for 30 min.
5. Wash with PBS (2 mL, 37°C), detach cells with 0.5 mL accutase (incubate for 7 min at 37°C).
6. Resuspend cells in culture medium (1 mL) and centrifuge for 5 min (20 g; 4 °C).
7. Wash cells with PBS (5 mL), repeat centrifugation, remove PBS.
8. Resuspend cells in PBS (0.5 mL), fix with cold 80% ethanol (4.5 mL; -20°C, dropwise with gentle shaking) and incubate on ice for 30 minutes.
9. Centrifuge cells, remove ethanol and resuspend in counterstaining solution (0.5 mL; 15 µg DAPI per 1 mL of 40% ethanol; 0.06% Triton X-100; 1% FBS in PBS).
10. Analyze cells by flow cytometry (DAPI: excitation 355 nm, emission 425-475 nm; area; linear; Cy3: excitation 561 nm, emission 575-590 nm).

Note: *Treatment time and concentration depend on cell type. Conditions for several cell types are given in reference 1.*

Rev.1.0/2019-07-11/SCT0464MD

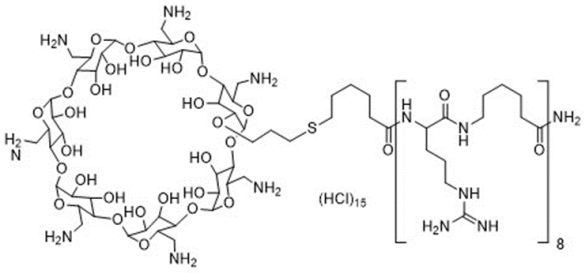

Figure 2. Chemical structure of the BioTracker NTP-Transporter molecule. The NTP-Transporter is a bifunctional molecule consisting of 1) a receptor site that forms a highly-stable non-covalent complex with a triphosphate moiety such as fluorescently labeled NTP (ie. Cy3-dUTP) and 2) a cell-penetrating agent (CPA) capable of carrying the whole complex across the plasma membrane lipid bilayer.

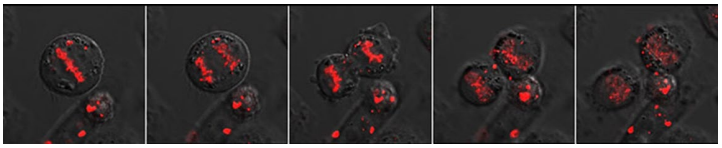

Figure 3. BioTracker NTP-Transporter labels mitotic cells. Confocal time-lapse live-cell imaging of mitosis of TZM-bl cells; the DNA was labeled with Cy3-dUTP. Cy3 fluorophore is represented with red color.

References

Zawad Z et al. *Transport of Nucleoside Triphosphates into Cells by Artificial Molecular Transporters.* Angew. Chem. Int. Ed. 2018. 57. 9891-9895.

Guixens-Gallardo, P. et al. *Brightly Fluorescent 2'-Deoxyribonucleoside Triphosphates Bearing Methylated Bodipy Fluorophore for in Cellulo Incorporation to DNA, Imaging and Flow Cytometry*. Bioconjugate Chem. 2018, 29, 3906-3912.