Data Sheet

BioTracker™ Far Red Live Cell Cycle Assay Solution

Live Cell Dye

SCT280

Pack Size: 1 vial of 50 tests

Store at –20 °C

FOR RESEARCH USE ONLY

Not for use in diagnostic procedures. Not for Human or Animal Consumption.

Background

Events in the cell cycle are integral to the critical phenomena of cell growth. Discrete phases of the cell cycle can be analyzed by staining DNA and using a flow cytometer. Propidium iodide (PI) is a reagent widely used for cell cycle analysis by flow cytometry. Unfortunately, PI is limited by the following:

- Cell fixation is required in order to use PI, as the cell membrane is not permeable to it
- RNase treatment is needed, as PI does not discriminate among nucleic acids
- Appropriate compensation is critical, as PI is detected by the PE channel and therefore overlaps if FITC, GFP or other ubiquitous green fluorescent reagents are used in the experiment.

The BioTracker™ Far Red and BioTracker™ Blue Cell Cycle Assay Solutions overcome the limitations that staining with PI can present. These solutions offer a much simpler procedure when compared with the steps necessary for using PI. Two fluorescent color options (far red and blue, SCT281) are provided as ready-to-use solutions. These reagents enable the determination of the ratio of the G0/G1 phase, S phase, and G2/M phase using a flow cytometer. Two fluorescent color options ensure compatibility and increase versatility in multiplex experiments.

Source

SCT280 and SCT281 do not contain genetically modified organisms.

Spectral Properties

SCT280: BioTracker™ Far Red Live Cell Cycle Assay Solution
Excitation peak: 640 nm
Emission peak: 780/60 nm

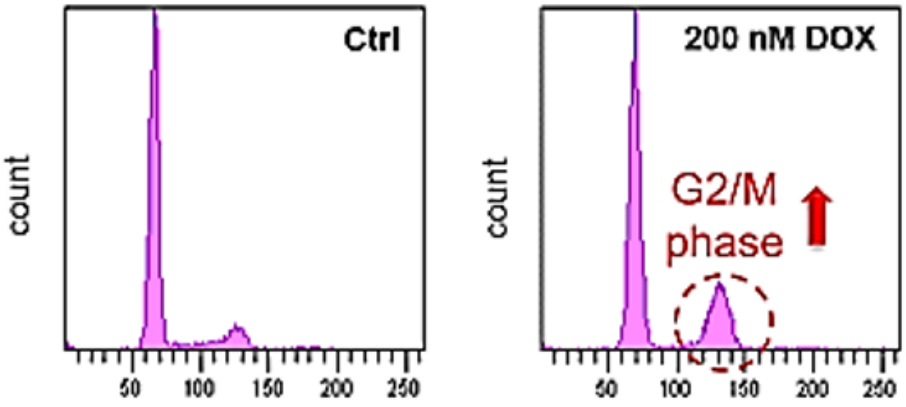
SCT281: BioTracker™ Blue Live Cell Cycle Assay Solution
Excitation peak: 405 nm
Emission peak: 450/50 nm

Storage and Handling

Store BioTracker™ Far Red Live Cell Cycle Assay Solution at –20 °C, protected from light.

Representative Data

Cell cycle changes following doxorubicin treatment:



Blue filter Blue filter

Protocols

Prepare BioTracker™ Live Cell Cycle Assay solution

1. Warm the vial to room temperature.
2. Before opening the vial, briefly centrifuge to collect solution at the bottom of the tube.

Protocol for staining live cells

1. Prepare a cell suspension (1-5 x 10^5 cells/mL) in a 1.5 mL microcentrifuge tube.
2. Centrifuge at 300 x *g* for 5 minutes and discard the supernatant.
3. To wash cells, add 500 µL of PBS to each microcentrifuge tube, suspend by pipetting, centrifuge at 300 × *g* for 5 minutes and discard the supernatant.
4. Add 500 µL of PBS to each microcentrifuge tube and add 5 µL of Cell Cycle Assay Solution. Protect from light.
5. Incubate in dark conditions at 37 °C for 15 minutes.
6. Pass through a cell strainer and analyze samples using a flow cytometer.

Protocol for staining fixed cells

1. Prepare a cell suspension (1-5 x 10^5 cells/mL) in a 1.5 mL microcentrifuge tube.
2. Centrifuge at 300 x *g* for 5 minutes and discard the supernatant.
3. Fix the cells by using one of the following protocols or your original fixation protocol.
4. Add 5 µL of Cell Cycle Assay solution and incubate at 37 °C for 15 minutes. Protect from light.
5. Pass through a cell strainer and analyze samples using a flow cytometer.

Fixation using a 4% paraformaldehyde (PFA)/PBS solution

1. Add 1 mL of a 4% PFA/PBS solution to a microcentrifuge tube and incubate at room temperature for 20 minutes.
2. Centrifuge at 300 x *g* for 5 minutes and discard the supernatant.
3. Add 500 µL of PBS to the microcentrifuge tube, suspend by pipetting, centrifuge at 300 x *g* for 5 minutes. Discard the supernatant and add 500 µL of PBS to each microcentrifuge tube.

Fixation using cold 70% ethanol

1. Add 1 mL of a 70% ethanol to a microcentrifuge tube and incubate at -20 °C for 120 minutes.
2. Centrifuge at 300 x *g* for 5 minutes and discard the supernatant.
3. Add 500 µL of PBS to the microcentrifuge tube, suspend by pipetting, centrifuge at 300 x *g* for 5 minutes. Discard the supernatant and add 500 µL of PBS to each microcentrifuge tube.

Flow cytometry analysis

1. Optimize gating after staining because scatter (FSC and SSC) are changed by the staining process with the Cell Cycle Assay Solution.
2. Residual trypsin may result in improper histogram during flow cytometric analysis. It is therefore essential to remove trypsin by washing before staining.
3. Too many cells may cause sample precipitation. Cell number/cell titer optimization is required.

Example flow cytometry filter settings for analysis of cells stained with Cell Cycle Assay Solution

Product	Excitation (nm)	Emission (nm)
SCT280	633–647 nm	695 LP or 780/60 BP
SCT281	405–407 nm	450/50 BP

Note: LP: Long pass filter, BP: Band pass filter

References

1. N. Sasaki, Y. Itakura and M. Toyoda. Rapamycin promotes endothelial-mesenchymal transition during stress-induced premature senescence through the activation of autophagy. Cell Commun. Signal, 2020. 18(1), 43.
2. T. Yamazaki, H. Suzuki, S. Yamada, K. Ohshio, M. Sugamata, T. Yamada and Y. Morita, *Lactobacillus paracasei* KW3110 Suppresses Inflammatory Stress-Induced Premature Cellular Senescence of Human Retinal Pigment Epithelium Cells and Reduces Ocular Disorders in Healthy Humans. Int J Mol Sci, 2020. 21(14), 5091.
3. N. Sasaki, F. Gomi, F. Hasegawa, K. Hirano, M. Fujiwara, M. Toyoda and T. Ishiwata. Characterization of the metastatic potential of the floating cell component of MIA PaCa-2, a human pancreatic cancer cell line. Biochem. Biophys. Res. Commun, 2020. 522(4), 881-888.

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