Data Sheet

LuminiCell Tracker™ 670- Cell Labeling Kit

SCT011

Pack Size: 1 Kit

Store at 2-8 °C

FOR RESEARCH USE ONLY

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

Background

Long-term noninvasive cell tracking by fluorescent probes and quantum dots is of great importance to life science and biomedical engineering. Current methods used to fluorescently tag cells have been limited by short signal duration, high background auto-fluorescence or lengthy molecular cloning manipulations using GFP.

LuminiCell Trackers™ are biocompatible organic fluorescent nanoparticles based on Aggregation Induced Emission (AIEdot) technology. Aggregation induced emission (AIE) molecules emit fluorescence in an opposite manner than other common fluorophores (Quantum Dots, GFP). Propeller-shaped AIE fluorogens are non-emissive in solutions but become highly fluorescent upon aggregate formation. Due to these differences, LuminiCell Trackers™ have very high fluorescence intensities with minimal signal quenching allowing live cell fluorescent tagging for up to 10 days *in vitro* and 21 days *in vivo*. These properties make them optimal candidates for long interval live cell bio-imaging experiments.

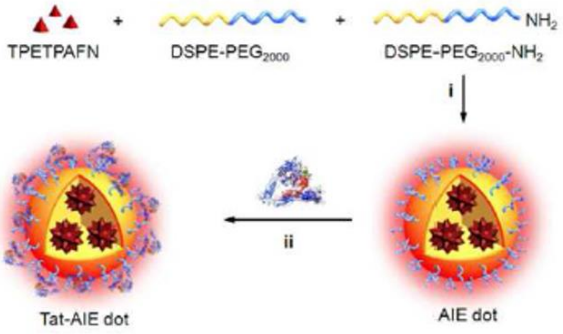

Figure 1. Fabrication of LuminiCell Tracker™ nanoparticles includes encapsulation of the TPETPAFN AIE molecules within a DSPE-PEG200 outer shell with attached cell permeable TAT sequences.

Quality Control Testing

Absorbance: 510 ±5 nm

Concentration: 180-220 nM

Fluorescence: 665 ±10 nm

Quantum Yield: ≥50%

Brightness at 670 nM: ≥1.7 X 10^7 $M^{-1}cm^{-1}$

Cellular Assay: HeLa Cell Fluorescence

Storage and Handling

Store at 2-8 °C upon receipt. Thaw at room temperature or in a water bath. Do not freeze.

Note: Some particulates may form as a result of nanoparticle aggregation during shipping. To get particulates back in solution, sonicate the vial containing LuminiCell Tracker™ three times for 1 min each before use.

Protocols

Product Information

Product Name	Concentration	Storage	Shelf-life	Absorption Maximum	Emission Maximum
LuminiCell Tracker™ 540	200 nM in 1X PBS, pH 7.4	2-8 °C	When store as instructed, stable for at least 6 months	423 nm	540 nm
LuminiCell Tracker™ 670	200 nM in 1X PBS, pH 7.4	2-8 °C		510 nm	670 nm

Compatible Instrument Parameters

Product Name	Laser Excitation (nm)	Filter (nm)
LuminiCell Tracker™ 540	405/458/488	480-560
LuminiCell Tracker™ 670	458/488/543	670-800

Labeling Adherent Cells

1. Culture cells in an 8-well Millicell® EZ slide (PEZGS0816) in a 5% CO_2 incubator at 37 °C.
2. When cells reach 80% confluence, remove the medium and wash cells once with 1X PBS.
3. Prepare the labeling solution at 2 nM working concentration by diluting the stock LuminiCell Tracker™ solution using fresh growth medium.

   Note: The working concentration is typically in the range of 2-10 nM depending on cell type and/or application requirements.
4. Add 0.2-0.4 mL of labeling solution into each well. For cells cultured on coverslips, pipet ~0.15 mL of labeling solution onto the cells grown on coverslips placed in a Petri dish.
5. Incubate cells in a 5% CO_2 incubator at 37 °C for ~1 hr.

   Note: Longer incubation (4-12 hrs) can be used to achieve higher uptake efficiency depending on applications.
6. Gently wash the cells twice with growth medium.
7. Visualize the labeled cells using any suitable fluorescence microscope or flow cytometry with compatible lasers/filters (refer to the table below for excitation and emission wavelengths of LuminiCell Trackers™).

   For fixed cell imaging, replace step 6 above as follows:
   a. Wash cells twice with 1X PBS and fix cells in 75% alcohol or 3.7% formaldehyde in PBS for 15 min.
   b. Wash cells twice after fixation prior to fluorescence imaging.

   For flow cytometry or applications that require cell detachment: Allow cells to recover in fresh growth medium for at 2 hours before detaching cells for flow cytometry or other applications.

Labeling Cells in Suspension

1. Prepare labeling solution at 2 nM working concentration by diluting the stock LuminiCell Tracker™ solution using fresh growth medium.

   Note: The working concentration is typically in the range of 2-10 nM depending on the cell type and/or application requirement.
2. Add 0.2-0.4 mL of labeling solution to a tube.
3. Add 1 × 10^6 cells from a cell suspension (vol ~0.1 mL) in growth medium into the tube containing the labeling solution.
4. Incubate cells in a 5% CO_2 incubator at 37 °C for ~ 1 hr.
5. Wash cells twice with growth medium.
6. Visualize the labeled cells using any suitable fluorescence microscope preferred by the user or flow cytometry with compatible lasers/filters (refer to the table below for excitation and emission wavelengths of LuminiCell Trackers™).

Representative Data

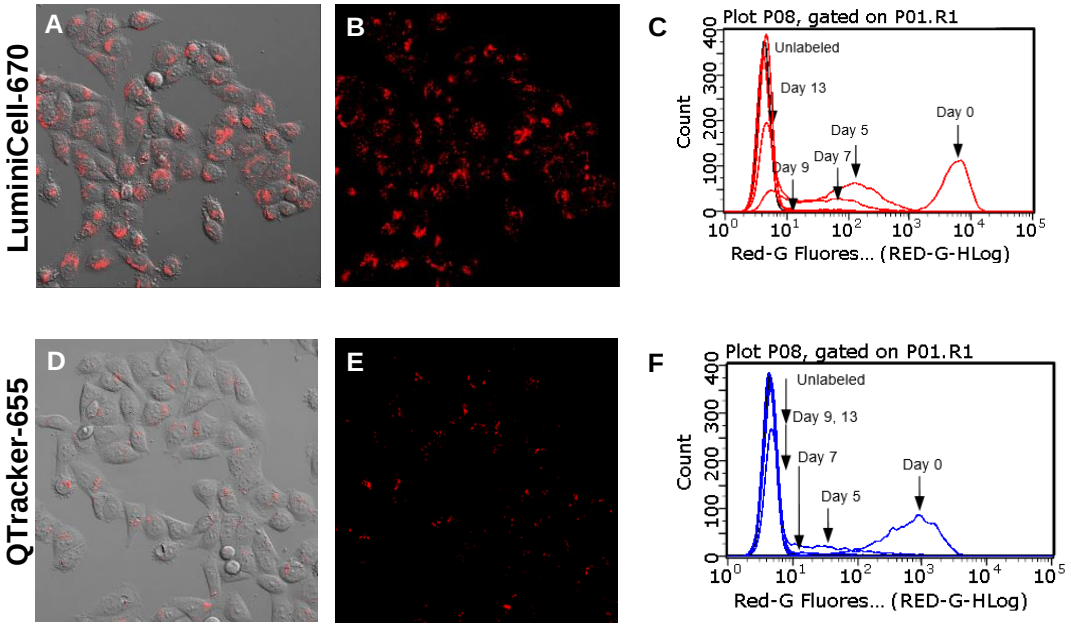

Figure 2: (**A**, **B**) LuminiCell Tracker™ 670 emits stronger signals (**C**) and retains the signal longer (**D**, **E**, **F**) than QTracker™ 655. (**C**, **F**) HeLa cells were plated at 500K cells per well of a 6-well plate overnight. Next day, 4 nM LuminiCell Tracker™ 670 or QTracker 655 were added and incubated for 4 hours and then imaged at Day 1. Cells were washed twice with PBS and incubated with fresh growth media for 2-3 hours to allow for cell recovery before being detached with Accutase®. Each cell suspension was diluted 1:2, 1:4, 1:8, 1:16 and 1:32, respectively, with growth medium and tracked for 13 days before imaging and flow analysis. The different dilution folds are necessary to make sure that there will be sufficient number of cells at the designated generation for imaging or flow cytometry. (**B**, **E**) Diluted cells were imaged at Day 1. (**A**, **D**) Fluorescent images were overlayed with brightfield images.

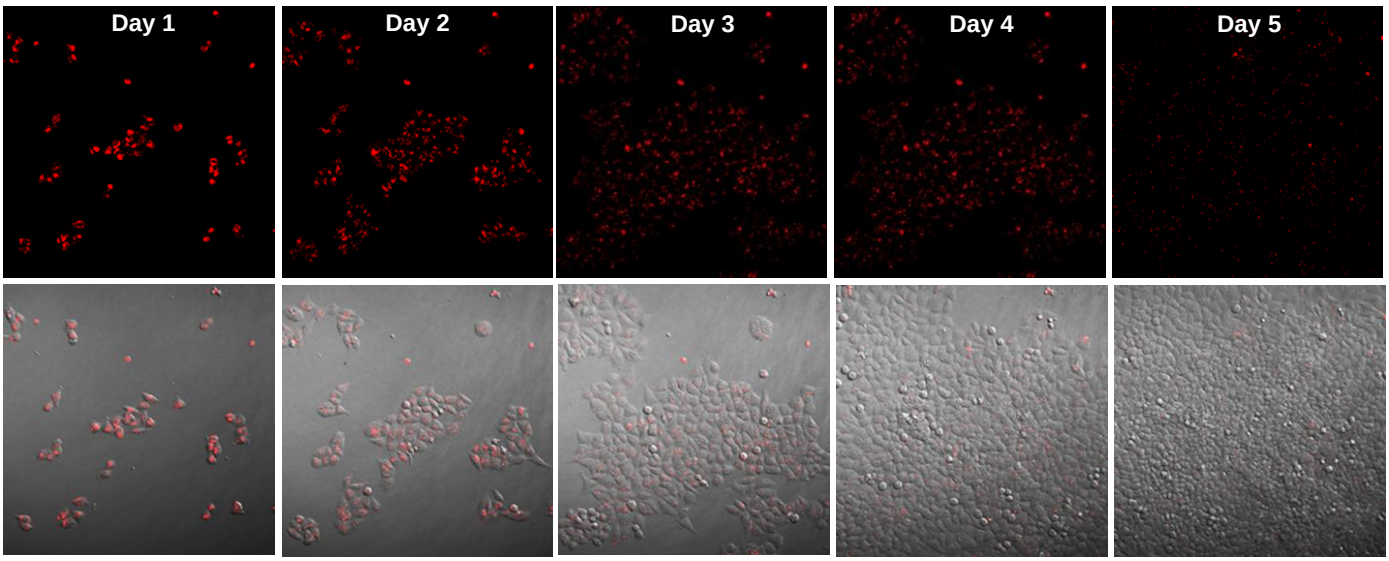

Figure 3. Real-time cell tracking of proliferating HeLa cells over 5 days. HeLa cells were labeled with 4 nM LuminiCell Tracker™ 670 following the "Labeling Adherent Cells" protocol. After 2-3 hours recovery, HeLa cells were detached and 2,500 cells were plated in a 8-well chamber slide and monitored over 5 days.

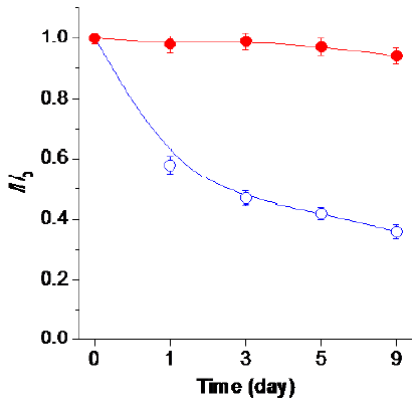

Figure 4: Time courses of photoluminescence intensity change of 2 nM LuminiCell Tracker™ 670 (red) in DMEM with 10% fetal bovine serum at 37 °C; data for quantum dots of QTracker® 655 (blue) are shown for comparison. I_o is the initial PL intensity, while I is the corresponding sample after the designated time interval.

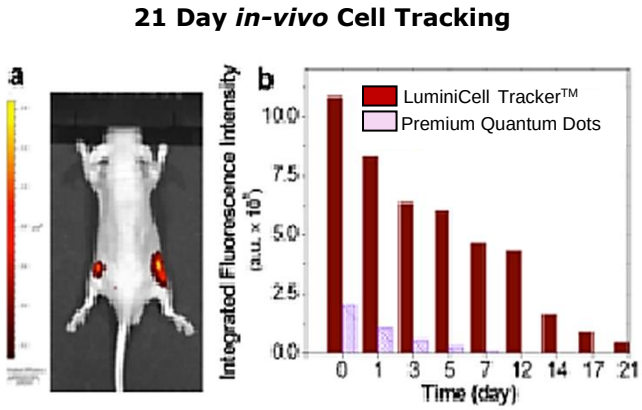

Figure 5: *In vivo* long-term tracking: 21 days *in vivo vs* 7 days for QD (after subcutaneous injection of labeled cancer cells.

References

1. Liu B, Tang BZ et al. Photostable fluorescent organic dots with aggregation-induced emission (AIE dots) for noninvasive long-term cell tracing. Sci Rep. 2013;3:1150.
2. Kang Y et al. Long-Term Tracking Mesenchymal Stem Cell Differentiation with Photostable Fluorescent Nanoparticles. ACS Appl Mater Interfaces. 2016 May 18;8(19):11925-33.

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