Long-term **Cell Tracer**

Name:	Long-term **Cell Tracer 675** Red, Cat.# P710R (1 ml), P710RS (0.1 ml)
	Long-term **Cell Tracer 580** Yellow, Cat.# P710Y (1 ml), P710YS (0.1 ml)
	Long-term **Cell Tracer 535** Green, Cat.# P710G (1 ml), P710GS (0.1 ml)
Application:	Cell Tracer is for long-term tracing of a wide range of cell types, including cancer cells, bone marrow stromal cell (BMSC), peripheral blood mononuclear cell, endothelial progenitor cell, human/mouse mesenchymal stem cells, skin stem cells *et al.* This product is for research use only.
Excitation / Emission:	See table below
Photostability:	*in vitro* tracing: 12 generations *in vivo* tracing: 3 weeks
Product Description:	**Cell Tracers** are a class of organic fluorescent dots with comparable size and photostability to inorganic quantum dots (QDs) to overcome the limitations of quantum dots (e.g., potential toxicity and compromised fluorescence in presence of ROS) in advanced bio-imaging applications. Upon conjugation with a cell penetrating peptide, Cell Tracer shows excellent labeling efficiency to living cells and **outperforms** the current gold standard inorganic quantum dots cell labeling reagents, in long term *in vitro / in vivo* cell tracing (**Fig. 1** & **Table 1.**). Stem cell tracing study also suggests that Cell Tracer has no negative effect on mesenchymal stem cell differentiation (**Table 2**). The merits of Cell Tracer make them promising alternatives to quantum dot probes, which is of high importance for translational research applications. Additionally, the biocompatible polymeric matrix endows this kind of organic fluorescent dots customized surface functional groups for further modification/conjugation with a variety of biomolecules for specific imaging tasks besides cell tracking (**Table 2**). ✓ **Long term photostability** - 3 weeks *in vivo* ✓ **High brightness** - high signal to noise ratio ✓ **Different colors** - blue, green, yellow, red ✓ **Low toxicity** - no heavy metal elements ✓ **Customization friendly** - can conjugate with desired biomolecules ✓ easy to use - only one component

	Excitation	Emission
Cell Tracer 675 Red	488nm or 532nm	675nm (650nm ~ 800nm)
Cell Tracer 580 Yellow	405nm or 488nm	580nm (550nm ~ 600nm)
Cell Tracer 535 Green	405nm	535nm (470nm ~ 600nm)

Shipping / Storage:	Ship at RT. Store at 4°C, — 20°C or — 80°C (Avoid repeated freeze-thaw cycles.)
Shelf Life:	1 months at RT, 3 months at 4°C, 12 months at — 20°C or — 80°C (preferred).
Component:	Cell Tracer 675 Red: P710R 1 mL; P710RS 0.1 mL Cell Tracer 580 Yellow: P710Y 1 mL; P710YS 0.1 mL Cell Tracer 535 Green: P710G 1 mL; P710GS 0.1 mL Concentration: 200 nM
Remark:	The different colors of Cell Tracers allow **simultaneously tracing** of two different groups of cells to investigate their migration and interaction.

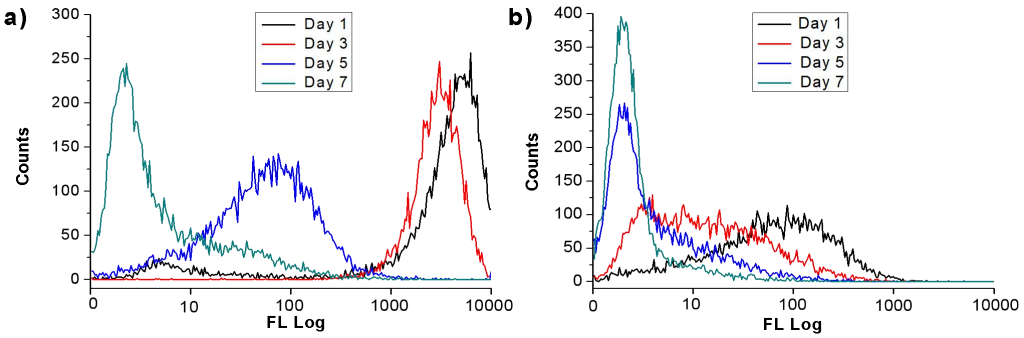


Figure 1.

Flow cytometry overlay histograms of MCF-7 cells at different time point after labeling with (**a**) 2 nM Cell Tracer or (**b**) 2 nM quantum dot.

Table 1. Cell Tracer outperforms other QDs in terms of long term tracing

	Day 1	Day 3	Day 5	Day 7
Cell Tracer	99.4%	98.2%	82.2%	31.1%
Other QDs	84.1%	43.9%	26.9%	4.3%

Table 1 summarizes the fluorescence intensity of the labeled cells at different time point from flow cytometry data in Fig. 1. These data show that Cell Tracer last much longer in labeled cells than other QDs.

Table 2. Comparison of Cell Tracer and other QDs

	Working concentration	Low Toxicity	Negative effect on stem cells	Customized targeting ability	Tracing ability

Cell Tracer	0.1 - 2 nM	√	√	√	9-12 generations
Other QDs	2 - 15 nM	×	×	×	5-6 generations

Cell Tracer has advantage over other QDs in many aspects including working concentration, toxicity and flexibility etc.

Protocol

The optimal working concentration of the Cell Tracer is typically in the range of 0.1 nM to 4 nM depending on the cell type and application. We recommend to test serial dilution test to figure the optimized staining condition for your cells. The following protocols use 2 nM Cell Tracer as example.

Make Cell Labeling Medium

Add 10 µL Cell Tracer (200 nM) to 1 mL complete cell culture medium, vortex for 30 seconds. Now the **Cell Labeling medium** containing 2 nM Cell Tracer is ready to use.

Always prepare the labeling medium freshly.

Labeling Adherent Cells (example of labeling in 6-well plate)

Plating cells:

Seed the cells in desired culture dish / flask. Cell density may vary depending on the cell type. Cells can be cultured on coverslip for special assay. The cells can be labeled when they attach and reach ~80% confluency. The time window could be a few hours to overnight.

Labeling:

1. Wash the cells twice using PBS.
2. Add 1 mL **cell labeling medium** into each well and incubate at 37 °C for 4 h to overnight.
3. Wash the cells twice with PBS.

   Optional: If desired, the labeled cells can be fixed at this point.
   Wash the cells 3 times with PBS, and then fix with 3.7% formaldehyde in PBS for 15 minutes at room temperature. Wash 3 times post-fixation in PBS prior to imaging.
4. The labeled cells are ready for further *in vivo* or *in vitro* assay.

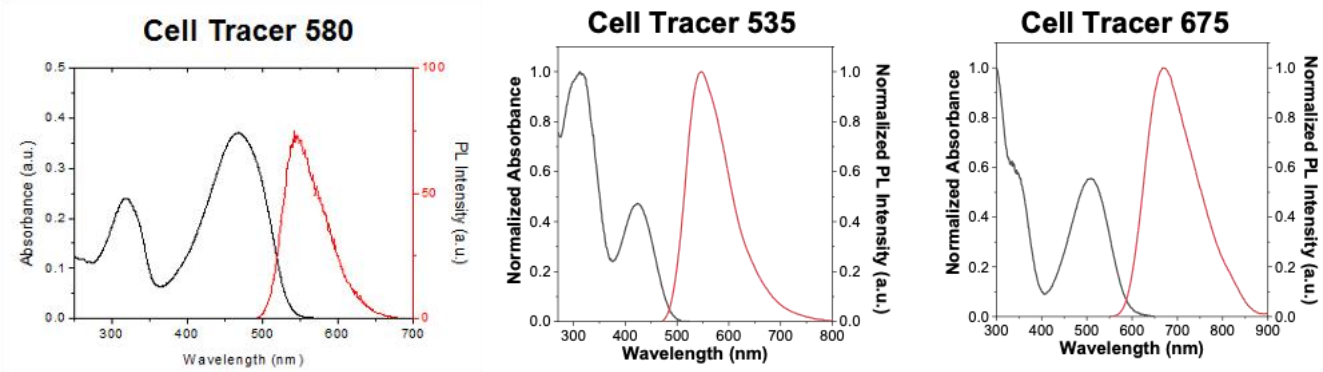
Labeling non-Adherent Cells

1. Collect the cells and centrifuge at 1500 rpm for 5 min. Discard the medium.
2. Add **cell labeling medium** to resuspend the cell pallet at 1 x 10^6 cells / 3 mL medium
3. Incubate at 37 °C for 4 hours.
4. Centrifuge at 1500 rpm for 5 min and wash the cells twice with PBS.

Optional: If desired, the labeled cells can be fixed at this point.

5. The labeled cells are ready for further *in vivo* or *in vitro* assay.

Fluorescence Spectrum:



Confocal imaging parameters:

If used separately

Cell Tracer 535 (Green): 405 nm excitation, 470-650 nm any bandpass or 520 nm above long pass filters.

Cell Tracer 580 (Yellow): 488 nm excitation, 505 nm above long pass filter.

Cell Tracer 675 (Red): 488 nm or 532 nm excitation, 600-800 nm any bandpass or 600 nm above long pass filters.

If use the two probes for two groups of cells to simultaneously perform dual-color cell tracking:

Cell Tracer 535 (Green): 405 nm excitation, 480-550 nm bandpass

Cell Tracer 675 (Red): 488 nm/532 nm excitation, 660-800 nm bandpass/700 nm above long pass filter.

For Confocal with tunable excitation (e.g., white light laser for Leica), one can choose 455 nm to excite both Cell Tracer 535 and 675 for dual-color cell imaging.

Carefully tune the confocal parameters, such as gain, laser power and bandpass filters, it will give two distinct signals from green and red tracers without crosstalking.

Note: The availability of filters depends on confocal microscopies, the operator always can try and find the optimized filters to obtain maximized signal.

-- The end –