molecular probes®
by life technologies™

Qtracker® Cell Labeling Kits

Table 1 Contents and storage

Material	Amount	Concentration	Storage	Stability
Qtracker® Cell Labeling Kits (Cat. nos. A10198, Q25001MP, Q25011MP, Q25021MP, Q25031MP, Q25041MP, Q25061MP, Q25071MP)				
Qtracker® nanocrystals (Component A)	100 µL	2 µM in 50 mM borate buffer, pH 8.3	• 2–8°C • DO NOT FREEZE	When stored as directed, the product is stable for at least 6 months.
Qtracker® carrier (Component B)	100 µL	Phosphate buffered saline (PBS), pH 7.2		
Qtracker® Cell Labeling Kits – Trial Size (Cat. nos. Q25029, Q25049, Q25069, Q25079)				
Qtracker® nanocrystals (Component A)	25 µL	2 µM in 50 mM borate buffer, pH 8.3	• 2–8°C • DO NOT FREEZE	When stored as directed, the product is stable for at least 6 months.
Qtracker® carrier (Component B)	25 µL	Phosphate buffered saline (PBS), pH 7.2		
Approximate fluorescence excitation and emission maxima: see Table 2, page 2.				

Introduction

Qtracker® Cell Labeling Kits are designed for loading cells grown in culture with highly fluorescent Qdot® nanocrystals. Once inside the cells, Qtracker® labels provide intense, stable fluorescence that can be traced through several generations, and are not transferred to adjacent cells in a population.

Qtracker® Cell Labeling Kits are available in seven colors—525 nm, 565 nm, 585 nm, 605 nm, 625 nm, 655 nm, 705 nm, or 800 nm emission—and are excellent tools for long-term tracking or imaging studies of live cells, including migration, motility, morphology, and other cell function assays.

The Qtracker® Cell Labeling Kits use a custom targeting peptide[1,2] to deliver Qdot® nanocrystals into the cytoplasm of live cells. Cytoplasmic delivery by this mechanism is not mediated by a specific enzyme; therefore, no cell-type specificity has been observed. Delivery is typically accomplished in less than 1 hour. Qdot® nanocrystals delivered by the Qtracker® Cell Labeling Kits are compatible with serum-sensitive cells; intense fluorescence is maintained in complex cellular environments and under various biological conditions including changes in intracellular pH, temperature, and metabolic activity. Furthermore, autofluorescence commonly observed in cells or tissues can be avoided using Qtracker® 655, 705, or 800 Kits.

Table 2 Fluorescence excitation and emission maxima for Qtracker® Cell Labeling Kits.

Product	Catalog no.	Emission (nm)	Excitation (nm)
Qtracker® 525 Cell Labeling Kit	Q25041MP and Q25049	525	405–485
Qtracker® 565 Cell Labeling Kit	Q25031MP	565	405–525
Qtracker® 585 Cell Labeling Kit	Q25011MP	585	405–545
Qtracker® 605 Cell Labeling Kit	Q25001MP	605	405–565
Qtracker® 625 Cell Labeling Kit	A10198	625	405–585
Qtracker® 655 Cell Labeling Kit	Q25021MP and Q25029	655	405–615
Qtracker® 705 Cell Labeling Kit	Q25061MP and Q25069	705	405–665
Qtracker® 800 Cell Labeling Kit	Q25071MP and Q25079	800	405–760
See Figure 5 for Qdot® nanocrystal excitation and emission spectra.			

Features and Applications

Using Qtracker® Cell Labeling Kits, you can observe labeled cells using extensive continuous illumination, without the photobleaching and degradation problems often associated with organic dyes.[3–5] Qtracker® labels are distributed in vesicles in the cytoplasm (Figure 1, below), and are inherited by daughter cells for at least six generations. Fluorescence from the Qtracker® labels can be seen up to a week after delivery in some cell lines. Long-term cellular retention makes Qtracker® Cell Labeling Kits ideal for studying cell motility (Figure 2, below), migration, differentiation, morphology, and many other cellular function studies.[3,4] Qtracker® labels do not leak out of intact cells to be taken up by adjacent cells in the population (Figure 3, page 3).

Figure 1 Distribution of Qtracker® labels in the cytoplasm: HeLa cells labeled with the Qtracker® 655 Cell Labeling Kit were observed with a Leica TCS SP2 confocal microscopy to see the distribution of the Qtracker® reagent in the cytoplasm (excitation at 488 nm).

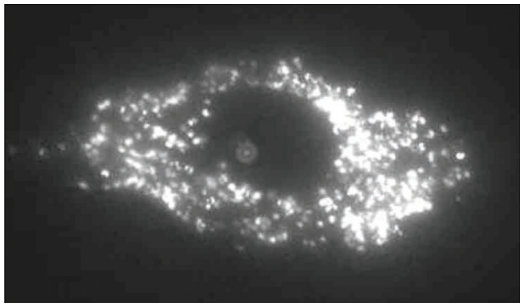


Figure 2 Motility of HeLa cells: A monolayer of HeLa cells was labeled with the Qtracker® 655 Cell Labeling Kit. The gap was made by scratching with a 200 µL pipette tip (~1 mm) and imaged using a Leica TCS SP2 confocal microscope (excitation at 488 nm; 10X objective). The cells moved to fill the gap and retained normal motility.

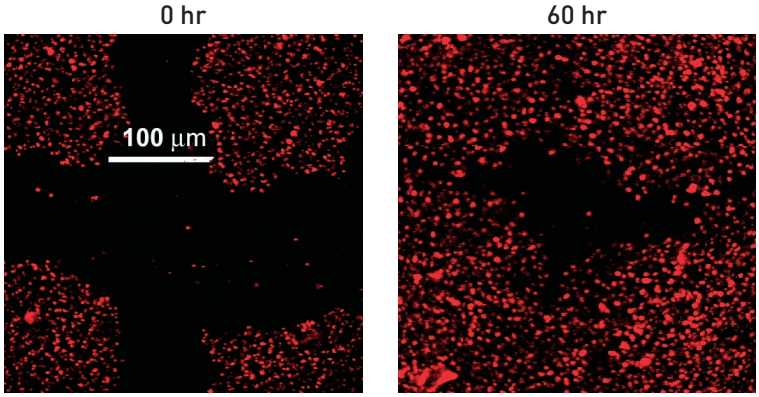
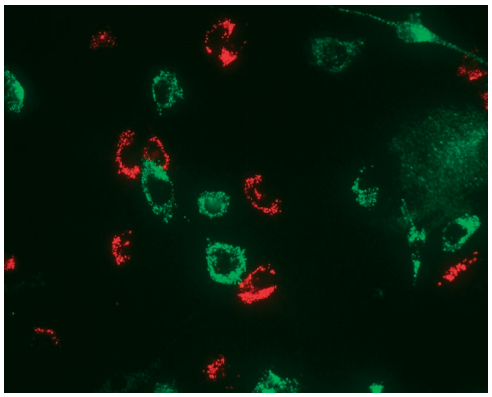


Figure 3 U-118 and HeLa cells were labeled with Qtracker® 565 and 655 Cell Labeling Kits, respectively, and co-cultured for 24 hours. The image was captured using a Leica TCS SP2 confocal microscope. Both colors are easily resolved, well retained, and well segregated in their respective cell lines.



Imaging Platforms

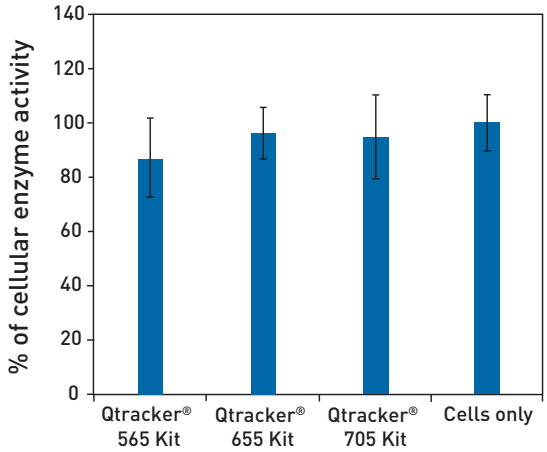
Qtracker® reagent-labeled live cells can be easily monitored on a variety of platforms, including flow cytometry, fluorescence/confocal microscopy, fluorescence microplate readers, and high-content imaging systems.

Cytotoxicity and Viability Studies

The cytotoxicity of the materials use in Qtracker® Cell Labeling Kits has been tested in a variety of cell lines including CHO, HeLa, U-118, 3T3, HUVEC, and Jurkat cells. Labeling with Qtracker® Cell Labeling Kits appears to exert minimal impact on cellular surface marker expression, cell proliferation, cellular enzyme activity, and cell motility; no effect on the CD3 expression level of Jurkat cells was observed following labeling with the Qtracker® Cell Labeling Kit.

The effect of Qdot® nanocrystal loading on cellular viability has been examined using cell proliferation and cellular enzyme activity measurements (Figure 4, below). The results indicate that labeling with the Qtracker® Cell Labeling Kit has no significant effect on cell proliferation and cellular enzyme activity.

Figure 4 Effect of Qtracker® labeling on cell viability: U-118 cells cultured in 96-well plates were labeled for 60 minutes with Qtracker® 565, 655, and 705 Kits. CellTiter 96 Non-Radioactive Cell Proliferation Assay (Promega) was performed on those cells 24 hours after labeling. The enzyme activity of unlabeled cells was used for normalization.



Cell Labeling with Qtracker® Cell Labeling Kits

Labeling efficiency of your cells with the Qtracker® Cell Labeling Kit of your choice (Table 2, page 2) can be tested using the basic protocols for suspension or cultured adherent cells described below. Post-labeling, researchers have demonstrated a wide variety of applications for Qtracker® labeled cells, including cell co-culture and cell assembly into heterotypic assemblies,[6] multilineage differentiation,[7] transdifferentiation versus cell fusion,[8] embryonic and mesenchymal stem cell tracking,[9–10] and cell migration dynamics.[12]

Labeling Suspension Cells

The basic protocols below have been tested using a limited number of representative cell types (CHO, HeLa, U-118, 3T3, HUVEC, and Jurkat cells). Optimization may be required for optimal labeling of your cells based on your initial results. For example, in Step 1.4 below, longer incubation times (2–24 hours) can be used for nanocrystal loading depending on experimental conditions and cell type. As part of optimization, we recommend performing cell viability tests (see Figure 4, page 3) using a standard cell proliferation assay method such as Vybrant® MTT Cell Proliferation Assay Kit (Cat. no. V13154).

Note

We recommend that you read the entire protocol before starting. For additional information, visit http://probes.invitrogen.com/products/qdot.

Materials Required but Not Provided

- Mammalian suspension cells of choice
- Complete growth medium for the cell type used
- Microcentrifuge tubes

Qtracker® Cell Labeling Kit Protocol for Suspension Cells

1.1 To prepare 10 nM labeling solution, pre-mix 1 µL each of Qtracker® Component A and Component B in a 1.5 mL microcentrifuge tube. Incubate for 5 minutes at room temperature and proceed immediately to Step 1.2.

Note: The working concentration of the Qtracker® label is typically in the range of 2 nM to 15 nM depending on the cell type and application. Prepare dilutions based on the 2 µM concentration of Qtracker® Component A. Scale volumes as appropriate for the number of suspension cell samples to be treated.

1.2 Add 0.2 mL of fresh complete growth medium to the tube and vortex for 30 seconds.

1.3 Add 1×10^6 cells (from a cell suspension at ~1×10^7 cells/mL in growth medium) to the tube containing the labeling solution.

1.4 Incubate the sample at 37ºC for 45–60 minutes.

1.5 Wash cells twice with complete growth medium.

1.6 Visualize labeled live cells using any suitable fluorescence microscopy method or flow cytometry with appropriate filters (see Table 2, page 2, for fluorescence excitation/emission details).

Labeling Adherent Cells

Materials Required but Not Provided

- Cell lines such as: HeLa cells (ATCC no. CCL-2) or U-118 cells (ATCC no. HTB-15)
- 8-well Lab-Tek chambered cover glass system or sterile coverslips suitable for subculturing cells in Petri dishes
- 75 cm^2 cell culture flask
- Optional: freshly-made 3.7% formaldehyde fixative solution (mix 1 mL 37–40% formaldehyde with 9 mL PBS)
- ATCC medium for HeLa: Minimum essential medium (Eagle) with 2 mM L-glutamine and Earle's BSS adjusted to contain 1.5 g/L sodium bicarbonate, 0.1 mM non-essential amino acids, and 1.0 mM sodium pyruvate, 10% fetal bovine serum
- ATCC medium for U-118: Dulbecco's modified Eagle's medium with 4 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate and 4.5 g/L glucose, 10% fetal bovine serum

Qtracker® Cell Labeling Kit Protocol for Adherent Cells

Subculture Cells

2.1 Subculture HeLa or U-118 cells from 75 cm^2 cell culture flasks in 8-well Lab-Tek chambered coverglass system at a density of 2×10^4 cells per well (cell density may vary if using a different size plate). Cells subcultured on coverslips in culture plates and grown to similar confluence can also be utilized for small numbers of tests.

2.2 Incubate the cells in a 37ºC, 5% CO_2 incubator overnight.

Labeling Procedure

3.1 To prepare 10 nM labeling solution, pre-mix 1 µL each of Qtracker® Component A and Component B in a 1.5 mL microcentrifuge tube. Incubate for 5 minutes at room temperature and proceed immediately to Step 3.2.

Note: The working concentration of the Qtracker® label is typically in the range of 2 nM to 15 nM depending on the cell type and application. Prepare dilutions based on the 2 µM concentration of Qtracker® Component A. Scale volumes as appropriate for the number of cell samples to be treated.

3.2 Add 0.2 mL of fresh complete growth medium to the tube and vortex for 30 seconds.

3.3 Add 0.2 mL of labeling solution to the well with cells. For labeling cells grown on coverslips, pipet ~0.15 mL labeling solution directly onto coverslips kept in a 60-mm Petri dish and cover.

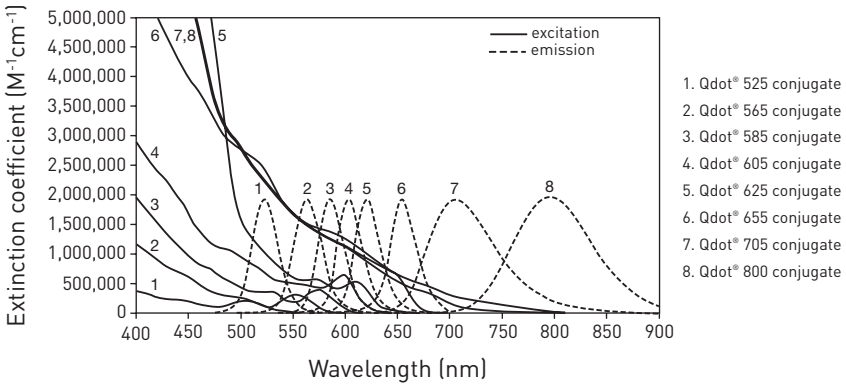
3.4 Incubate at 37ºC for 45–60 minutes.

3.5 Wash cells twice with complete growth medium.

Note: If desired, fix labeled cells at this point by washing 3 times with PBS, incubating with 3.7% formaldehyde in PBS for 15 minutes at room temperature, and washing 3 times post-fixation in PBS prior to imaging.

3.6 Visualize labeled live cells using any suitable fluorescence microscopy method or flow cytometry with appropriate filters (see Table 2, page 2, for fluorescence excitation/emission details).

Figure 5 Typical absorption and emission spectra of Qdot® 525 conjugate (1), Qdot® 565 conjugate (2), Qdot® 585 conjugate (3), Qdot® 605 conjugate (4), Qdot® 625 conjugate (5), Qdot® 655 conjugate (6), Qdot® 705 conjugate (7), Qdot® 800 conjugate (8).



Stem Cell Results

We have demonstrated the usefulness of a co-culture/cell separation strategy in stem cell work by labeling mouse embryonic fibroblasts (MEFs) with the Qtracker® 655 Cell Labeling Kit and culturing the cells with SA2p12 hESCs or BG1vp22 human embryonic stem cells (hESCs). Flow cytometry analysis of MEF cells transfected with Qtracker® 655 showed 97% efficiency in labeling (Figure 6, below). MEFs labeled with Qtracker® are easily discriminated from colonies of BG1vp22 hESCs (Figure 7, page 7) and suspensions of SA2p12 hESCs (Figure 8, page 7). In fact, hESC colonies appear to exclude the feeder cells, rather than grow on top of them. Furthermore, excellent separation of hESCs and MEFs was obtained by flow cytometry (Figure 9, page 7). These findings illustrate the usefulness of Qtracker® kits for labeling live feeder cells and discriminating them from co-cultured hESCs.

Figure 6 Flow cytometry analysis of MEFs transfected with Qtracker® 655 Cell Labeling Kit shows 97% labeling efficiency. Samples were analyzed on a FACS Canto™ (Becton Dickinson).

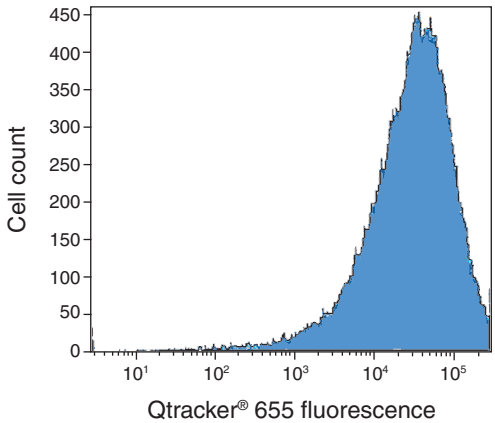


Figure 7 Mouse embryonic fibroblasts (MEFs) labeled with Qtracker® kits are easily discriminated from colonies of BG1vp22 human embryonic stem cells (hESCs). Colony of Oct 4-expressing BG1vp22 hESCs (Green; labeled with Alexa Fluor® 488 goat anti-rabbit IgG – Cat. no. A11034) co-cultured with Qtracker® 655 labeled MEFs (Red), and counterstained with DAPI (Blue). Samples were imaged using a Nikon® Eclipse® TE300. Image capture was done using a Nuance™ multispectral imaging system.

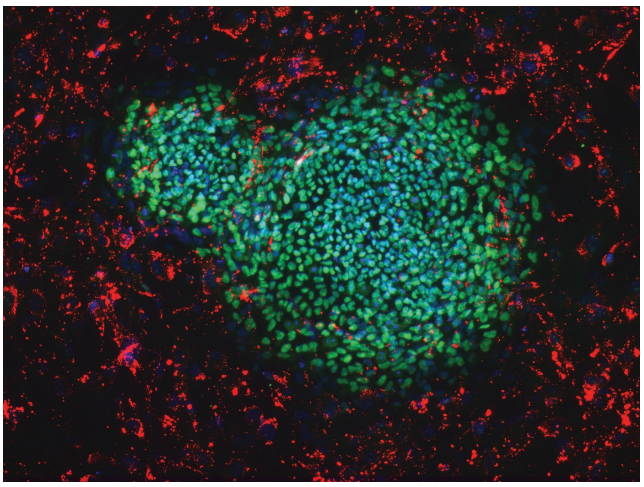


Figure 8 Discrimination of MEFs labeled with Qtracker nanocrystals from suspensions of SA2p12 hESCs. Suspension of Tra-1-81 expressing SA2p12 hESCs (Green; labeled with Alexa Fluor®488 goat anti-mouse IgM – Cat. no. A21042) co-cultured with Qtracker® 655 labeled MEFs (Red). Samples were imaged using a Nikon® Eclipse® TE300. Image capture was done using a Nuance™ multispectral imaging system.

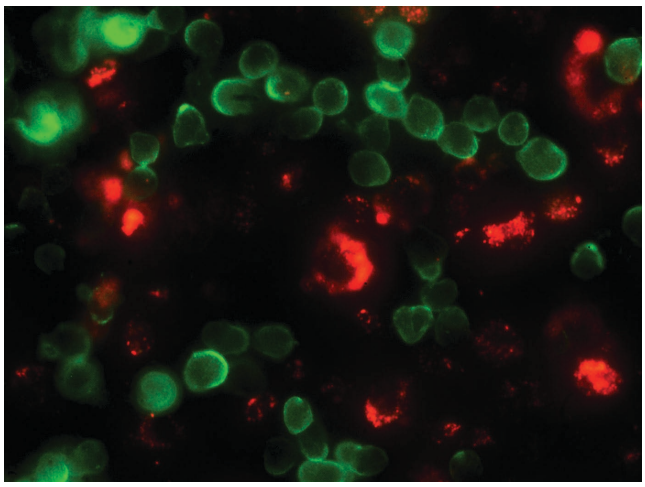
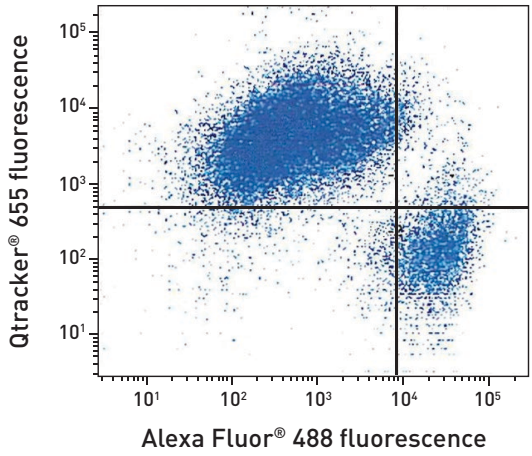


Figure 9 Separation of hESCs and MEFs by flow cytometry: Flow cytometry separation of SSEA4 expressing BG01vp29 cells (labeled with Alexa Fluor® 488 goat anti-mouse IgG3 (γ3), Cat. no. A21151) from Qtracker® 655 labeled cells. Due to the brightness of Qtracker® 655 labeled signals compared to Alexa Fluor® 488 dye signals, TransFluoSpheres® streptavidin-labeled microspheres, 0.04 µm (488/465) (Cat. no. T10711) was used to better match scales.



References

1. Curr Protein Pept Sci 4, 87 (2003); **2.** J Am Chem Soc 124, 368 (2002); **3.** Nat Biotech 21, 47 (2003); **4.** Biochem Biophys Res Comm 302, 496 (2003); **5.** Nano Lett 4, 2019 (2004); **6.** Am J Pathology 168, 1793 (2006); **7.** Stem Cells 25, 2760 (2007); **8.** Arterioscler Thromb Vasc Biol 25, 1388 (2005); **9.** BMC Biotechnol 7,67 (2007); **10.** J Nanobiotechnology 5, 9 (2007); **11.** Stem Cells 25, 2128 (2007); **12.** Cytotechnology 51, 7 (2006).

Product List

Current prices may be obtained from our website or from our Customer Service Department.

Cat. no.	Product Name	Unit Size
Q25041MP	Qtracker® 525 Cell Labeling Kit	1 kit
Q25031MP	Qtracker® 565 Cell Labeling Kit	1 kit
Q25011MP	Qtracker® 585 Cell Labeling Kit	1 kit
Q25001MP	Qtracker® 605 Cell Labeling Kit	1 kit
A10198	Qtracker® 625 Cell Labeling Kit	1 kit
Q25021MP	Qtracker® 655 Cell Labeling Kit	1 kit
Q25061MP	Qtracker® 705 Cell Labeling Kit	1 kit
Q25071MP	Qtracker® 800 Cell Labeling Kit	1 kit
Q25049	Qtracker® 525 Cell Labeling Kit *trial size*	1 kit
Q25029	Qtracker® 655 Cell Labeling Kit *trial size*	1 kit
Q25069	Qtracker® 705 Cell Labeling Kit *trial size*	1 kit
Q25079	Qtracker® 800 Cell Labeling Kit *trial size*	1 kit
Related Products		
A11034	Alexa Fluor® 488 goat anti-rabbit IgG	0.5 mL
A21042	Alexa Fluor® 488 goat anti-mouse IgM	250 µL
A21151	Alexa Fluor® 488 goat anti-mouse IgG3 (γ3)	250 µL
T10711	TransFluoSpheres® streptavidin-labeled microspheres	0.4 mL
V13154	Vybrant® MTT Cell Proliferation Assay Kit	1 kit

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