Track and separate MEFs within a co-culture of hESCs with Qdot® nanocrystals

Human embryonic stem cells (hESCs) require culture under conditions that prevent differentiation. Co-culturing hESCs with mitotically inactive mouse embryonic fibroblasts (MEFs) as feeder cells is commonly practiced to maintain hESC potency. This practice, however, presents challenges during subsequent physical isolation of hESCs without MEF contamination.

A useful cell separation strategy would utilize a long-lasting, live-cell label that does not compromise viability and allows identification and separation of live and or fixed cell preparations. The Qtracker® Cell Labeling Kits provide a simple and efficient method for fluorescently labeling MEFs with Qdot® nanocrystals for easy identification and subsequent separation of MEFs from hESCs.

Qtracker® Cell Labeling Kit reagents deliver fluorescent Qdot® nanocrystals into the cytoplasm using a custom targeting peptide. Once inside, Qtracker® labels provide intense, stable fluores-cence that can be observed up to 5 weeks after transfection. They are not transferred to adjacent cells in a population. Qtracker® Cell Labeling Kits are available with Qdot® nanocrystals in seven brilliant colors—525 nm, 565 nm, 585 nm, 605 nm, 655 nm, 705 nm, or 800 nm emission.

We demonstrated the usefulness of this co-culture/cell separation strategy by labeling MEFs with the Qtracker® 655 Cell Labeling Kit and culturing the cells with either SA2p12 hESCs or BG1vp22 hESCs. Flow cytometry analysis of MEF cells transfected with Qtracker® 655 showed 97% efficiency in labeling (Figure A). MEFs labeled with Qtracker® are easily discriminated from colonies of BG1vp22 hESCs (Figure B) and suspensions of SA2p12 hESCs (Figure C). 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 D). These findings illustrate the usefulness of Qtracker® kits for labeling live feeder cells and discriminating them from co-cultured hESCs.
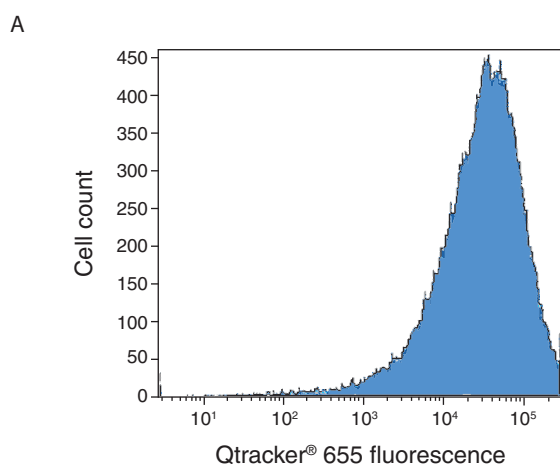
A

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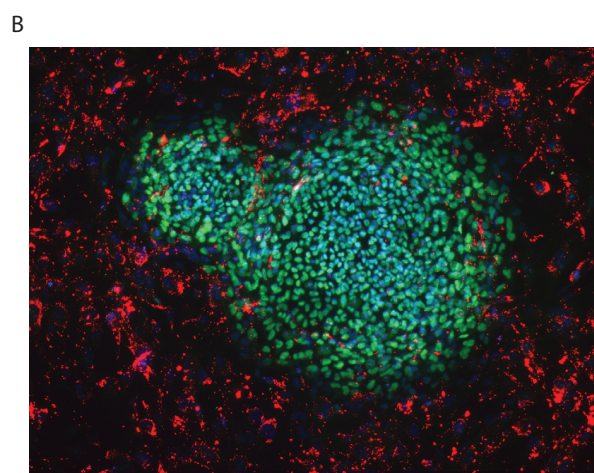
B

Figure B—MEFs labeled with Qtracker® kits are easily discriminated from colonies of BG1vp22 hESCs. Colony of Oct 4-expressing BG1vp22 hESCs (Green; labeled with Alexa Fluor® 488 goat anti-rabbit IgG – Cat. No. A-11034) 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.

ᴥ invitrogen™

C

D

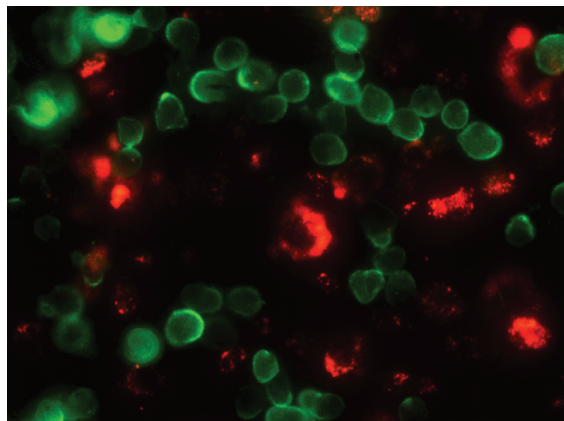Qtracker® 655 fluorescence

Alexa Fluor® 488 fluorescence

Figure C—MEFs labeled with Qtracker® kits are easily discriminated 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. A-21042) 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.
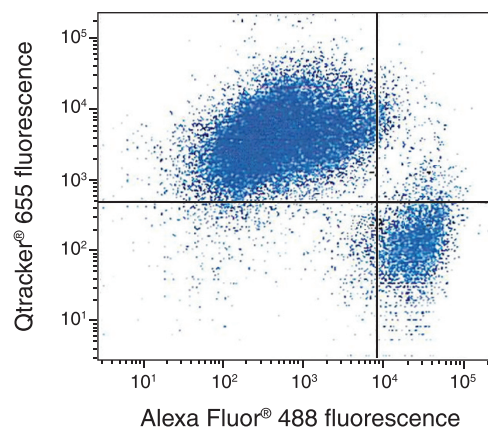
Figure D—Separation of hESCs and MEFs by Flow Cytometry. Flow cytometry separation of SSEA4 expressing BG01vp29 cells (labeled with Alexa Fluor® 488 goat anti-mouse IgG$_3$ (γ3) - Cat. No. A-21151) from Qtracker® 655 labeled cells. Due to the brightness of Qtracker® 655 signals compared to Alexa Fluor® 488 dye signals, TransFluoSpheres® streptavidin-labeled microspheres, 0.04 μm (488/645) (Cat. no. T-10711) was used to better match scales.

Ordering information

Product	Quantity	Cat. no.
Qtracker® 525 Cell Labeling Kit	1 kit	Q25041MP
Qtracker® 565 Cell Labeling Kit	1 kit	Q25031MP
Qtracker® 585 Cell Labeling Kit	1 kit	Q25011MP
Qtracker® 605 Cell Labeling Kit	1 kit	Q25001MP
Qtracker® 655 Cell Labeling Kit	1 kit	Q25021MP
Qtracker® 705 Cell Labeling Kit	1 kit	Q25061MP
Qtracker® 800 Cell Labeling Kit	1 kit	Q25071MP

Visit us at www.invitrogen.com/stemcell for a complete review of Invitrogen stem cell technologies and services.

invitrogen™

www.invitrogen.com