Epi-Fluorescence Mitochondrial Imaging in Live Single Cells and Tumorspheres

PromoCell

Application Note

Background

Mitochondria are rod-shaped organelles that generate most of a cell's supply of adenosine triphosphate (ATP), which is a source of chemical energy for executing various cellular processes (Campbell NA, Williamson B, Heyden RJ 2006). Mitochondria are also involved in crucial cellular tasks such as cell signaling, differentiation, and death, in addition to controilling the cell cycle and cell growth (McBride HM et al. 2006). Damage to and subsequent dysfunction of mitochondria play a role in a range of human diseases including diabetes, myopathy and other systemic disorders.

Staining of mitochondria with fluorescent dyes, antibodies or fluorescent molecules can greatly facilitate studies of their function and distribution and the viability of cells in healthy and diseased individuals.

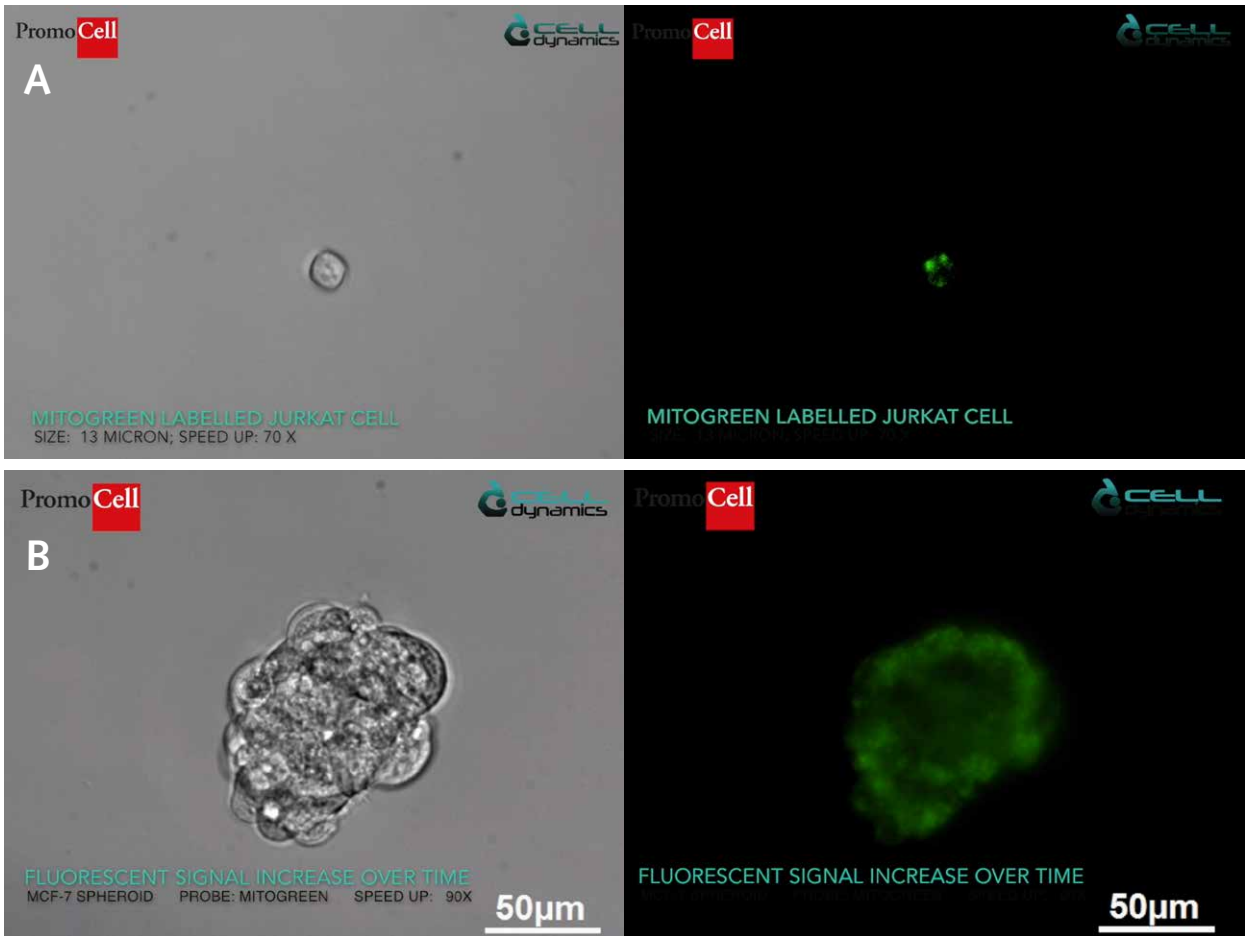

Fig. 1: Jurkat cell (A) and MCF-7 tumorsphere (B) cultured in 3D Tumorsphere Medium XF. Bright field image and green fluorescent microscopy (488 nm/ 523 nm). Cells were cultured in suspension and monitored in the CELLviewer (5% CO_2). Each experiment was conducted over 24 hours with a frame rate of 30 Hz and a field of view of 85 x 85 x 30 µm.

Watch a live imaging video of Jurkat cells and MCF-7 tumorspheres on our YouTube Channel:

Jurkat cell video https://youtu.be/gM8aSujDiuo

MCF-7 tumorsphere video https://youtu.be/WWMVUPsh-48

PromoCell's MitoGreen

MitoGreen is a green fluorescent mitochondrial dye with properties similar to those of MitoTracker Green FM. It evenly diffuses across the plasma membrane of live and fixed cells and accumulates in their mitochondria. The dye is nonfluorescent until it enters the mitochondrial compartment, with the mitochondrial mass affecting the intensity of fluorescence. Mitochondria stained with MitoGreen become highly fluorescent and can be used for both live and formaldehyde fixed cells. Blue and red markers are also available.

PromoCell's 3D Tumorsphere Medium XF

PromoCell 3D Tumorsphere Medium XF is a culture system developed for standardized serial culture of cancer cell lines as tumorspheres/mammospheres. It supports sustained cell proliferation and serial passage of the 3D culture. The serum-and xeno-free formulation provides a culture environment devoid of all stimuli from undefined materials.

CellDynamics CELLviewer

The CELLviewer system enables high-content, time-lapse fluorescence imaging of live cells in suspension. The cells can be grown in a cartridge that lets drugs or components be dispensed into the sample chamber for visualizing effects on single cells or multicellular/tumorsphere specimens.

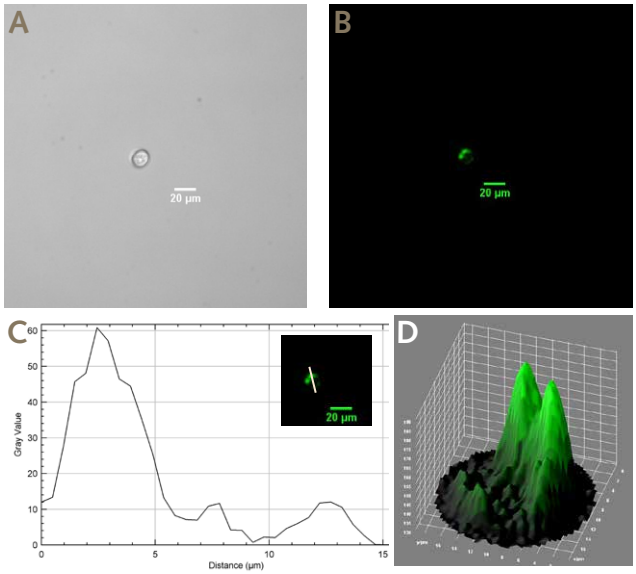

Fig. 2: Single Jurkat cell MitoGreen labelled analysis.
A: CELLviewer acquisition in bright-field channel.
B: CELLviewer acquisition in GFP channel.
C: 3D surface plot of MitoGreen (PromoCell) fluorescence signal with ImageJ software.
D: Plot profile of Mitogreen (PromoCell) fluorescence signal along a straight line crossing the cell. Scale bar: 20 µm

Note: Single-cell Jurkat cells and MCF7 tumorspheres were isolated and imaged for 4 and 7 hours respectively. Intensified labeling of the mitochondria and fluidic transport were observed over time. Primary and cell line imaging are possible in the CELLviewer setup.

Use aseptic techniques and a laminar flow bench.

A) Protocol for mitochondrial imaging

I. Materials

Mitochondrial Imaging Materials

- Jurkat cells (ATCC)
- Human cell culture or tumorsphere in good condition
- 3D Tumorsphere Medium XF (C-28070 or C-28075)
- MitoGreen (PK-CA707-70054)
- RPMI culture medium (Gibco, Life Technologies, Thermo Fisher Scientific)
- Cell culture vessel, e.g. disposable cartridge
- CELLviewer imaging system
- CELLviewer 50 ml DOCK

II. Protocol for single cells

Single Cell Mitochondrial Imaging Protocol

1. **Incubation of cells with MitoGreen**
   Jurkat cells were grown at 37°C and 5% CO_2 in RPMI 1640 medium, supplemented with 2 mM L-glutamine, 10% FBS, 100 units/mL penicillin and 100 mg/mL streptomycin. Before the experiments, Jurkat cells were washed and suspended at a final concentration of 5 x 105 cells/ml in a 5% FBS culture medium. The sample was then incubated for 20 minutes in the dark at 37°C with MitoGreen 200 mM.

2. **Sample preparation for CELLviewer imaging**
   After incubation, cells were centrifuged at 2000 rpm for 5 minutes to remove excess MitoGreen and resuspended in 5% culture medium at the CELLviewer working concentration of 5 x 103 cells/ml. The sample was then pipetted inside a 50 mL Falcon tube closed with a CELLviewer 50 ml DOCK. After single cell isolation and fluidic adaptive autofocusing, CELLviewer automatically acquired sample images in the bright-field channel and GFP channels at 0.5 fps with 20X magnification.

3. **Single cell analysis in CELLviewer**
   ImageJ software was used for image analysis using (i) the measuring function to determine the diameter of individual cells, (ii) a plot profile plugin for creating a fluorescence intensity along a straight line passing across the cell; and (iii) a 3D surface plot plugin for visualizing the distribution of spatial fluorescence in 3D.

III. Protocol for tumorspheres

Tumorsphere Mitochondrial Imaging Protocol

1. **Preparation of tumorspheres and MitoGreen**
   When cells exhibit an appropriate confluence, remove the medium and add pre-warmed medium containing 100 nM of the Mito Dye. For suspension cells, pellet the cells and resuspend in medium containing diluted Mito Dye.

   Note: The optimal staining concentration may vary depending on the cell type and application. We recommend performing an initial test with the dyes at concentrations between 20 and 200 nm. At higher concentrations, other structures may be stained.

   Note: Alternatively, the dye can be added directly to the culture medium. We recommend making a dilute stock solution in culture medium to avoid exposing

Use aseptic techniques and a laminar flow bench.

the cells to a transient high dye concentration. For example, dilute the Mito Dye to 10 times the final desired concentration in culture medium, and then add 1/10 volume of the dilute stock to the medium on the cells and mix well by gently pipetting up and down.

2. **Incubation of tumorspheres with MitoGreen**
   Incubate cells for 15 minutes or longer at 37°C. Washing is not required before imaging.
   Note: Longer staining times may result in brighter staining. Our various Mito Dyes exhibit no obvious toxicity at 100 nM in MCF-7 cells with incubation times up to 72 hours, but toxicity may vary according to the cell type.
3. **Tumorsphere analysis in CELLviewer**
   Analyze fluorescence by fluorescence microscopy or flow cytometry using the appropriate excitation/emission settings or detection channel (see Spectral Properties).
   Note: Our Mito Dyes are not well-retained after fixation. For fixed cell staining with MitoGreen, we recommend fixation before staining (see below). Other Mito Dyes cannot be used with fixed cells.

Tumorsphere Mitochondrial Imaging Protocol

Staining of fixed cells (MitoGreen only)

1. Fix cells in 4% paraformaldehyde in PBS (Cat. No. PK-CA707-22023) for 10 minutes at room temperature.

2. Following fixation, rinse cells in PBS and incubate with MitoGreen.

3. Rinse cells with PBS before imaging.

Staining Protocol

III. Analysis and results

For the image analysis the ImageJ software was used to compute:

- Single cell diameter
- Fluorescence intensity graphic (ImageJ profile plugin)
- 3D spatial fluorescnece intensity distribution (ImageJ 3D surface plugin)

Mitochondrial Imaging Analysis

Products

Product	Size	Catalog Number
3D Tumorsphere Medium XF	250 ml	C-28070
MitoGreen (Green-fluorescent mitochondrial dye)	20 x 50 µg	PK-CA707-70054

Related Products

Product	Size	Catalog Number
Primary Cancer Culture System	250 ml	C-28081
Cancer Cell Line Medium XF	250 ml	C-28077
Dulbecco's PBS, w/o Ca^{++}/Mg^{++}	500 ml	C-40232
DetachKit	3 x 125 ml	C-41210
Cryo-SFM	30 ml / 125 ml	C-29910 / C-29912
MitoBlue (Blue-fluorescent mitochondrial dye)	20 x 50 µg	PK-CA707-70052
MitoRed (Red-fluorescent mitochondrial dye)	20 x 50 µg	PK-CA707-70055
MitoNIR (Mitochondrial near-infrared dye for live cell staining)	20 x 50 µg	PK-CA707-70068
MitoFR (Mitochondrial far-red dye for live cell staining)	20 x 50 µg	PK-CA707-70075
Live/Dead Cell Staining Kit I (Calcein/EthD-III)	500 assays	PK-CA577-K315
Live/Dead Cell Staining Kit II (CFSR/7-AAD)	300-1000 assays	PK-CA707-30002
5(6)-CFDA, SE (CFSE)	25 mg	PK-CA707-90041
Calcein AM	1 mg	PK-CA707-80011
Calcein AM Solution, 4 mM in DMSO	100 µl	PK-CA707-80011-1
Calcein AM Solution, 1 mg/ml in anhydrous DMSO	1 ml	PK-CA707-80011-2
Calcein AM (special packaging)	20 x 50 µg	PK-CA707-80011-3
BCECF AM Solution	1 ml	PK-CA707-51009
BCECF AM	20 x 50 µg	PK-CA707-51011-1
BCECF AM	1 mg	PK-CA707-51012
5-(and-6)-Carboxy-2',7'-dichlorofluorescein diacetate	100 mg	PK-CA707-51016

References

1 Campbell NA, Williamson B, Heyden RJ 2006, *Biology: exploring life.* Pearson Prentice Hall.

2 McBride HM, Neuspiel M, Wasiak S 2006, *Mitochondria: more than just a powerhouse.* Curr Biol, 16(14): p. 551-560. doi: 10.1016/j.cub.2006.06.054.

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