## In Vitro Application

# APPLICATION NOTE:

# Fluorescent Triple Staining Protocol for LifeGel-Cultured Cells (DiO, MitoLite, Hoechst).

### DESCRIPTION

- **DiO (3,3'-Dioctadecyloxacarbocyanine Perchlorate)** – membrane dye
  DiO is a lipophilic fluorescent dye that integrates into lipid bilayers, making it ideal for staining cell membranes. It emits green fluorescence and is commonly used to trace cells, assess membrane integrity, or track cell migration in live or fixed samples.
  **Excitation/Emission:** ~484 / 501 nm

- **MitoLite™** (e.g., MitoLite Green/Red/Orange) – mitochondrial dye
  MitoLite dyes are specific mitochondrial stains used to visualize and monitor mitochondria in live cells. These dyes accumulate in active mitochondria based on membrane potential, providing insights into mitochondrial health and function. Different variants exist with various spectral properties.
  **Excitation/Emission:** Depends on variant (e.g., MitoLite Green ~488/515 nm)

- **Hoechst (e.g., Hoechst 33342) -** Nuclear dye (DNA-binding)
  Hoechst dyes are blue-fluorescent stains that bind to the minor groove of DNA, preferentially at A-T rich regions. Hoechst 33342 is cell-permeable and widely used to stain nuclei in live or fixed cells.
  **Excitation/Emission:** ~350 / 461 nm

REAL RESEARCH

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## In Vitro Application

# PROTOCOL:

# Fluorescent Triple Staining Protocol for LifeGel-Cultured Cells (DiO, MitoLite, Hoechst).

**Materials required:**

- PANC-1 and HEK293 cells 3D culture on LifeGel Plates (see protocol: Preparing LifeGel Plates)
- Culture medium with and without FBS (e.g., DMEM +/- 10% FBS + antibiotics)
- DiO
- MitoLite
- Hoechst
- Hank's Balanced Salt Solution
- 20 mM HEPES
- Pipette/multichannel pipettor
- Fluorescent microscope

Experiments were performed on a 96-well plate containing LifeGel with HEK293 and PANC-1 cells, which had already been growing for 2 weeks.

**PRIOR TO ASSAY -** 48 hours before the main experiment, perform staining of cells with DiO.

- Incubate cells in working solution (50 µL of dye per mL of serum-free medium) for 30 minutes.
- Carefully wash LifeGel/cell-containing wells 3 times with complete medium.

**ON THE DAY OF THE ASSAY** - prepare working solution of MitoLite and Hoechst.

- Add 20 µL of MitoLite stock solution to 10 mL of Hanks balanced salt solution (HBSS) with 20 mM HEPES (creating HHBS).
- To obtaine MitoLite and Hoechst staining solution add Hoechst to MitoLite working solution (final concentration of Hoechst in staining solution should be10 µg/mL).
- Before staining, carefully wash LifeGel/cell-containing wells twice with HBSS.
- Add 100 µL of staining solution per well (mix of MitoLite and Hoechst).
- Incubate cells for 60 minutes at 37 ºC.
- Wash twice with HBSS and leave in complete DMEM.
- Take images of fluorescence and brightfied using fluorescent microscope with different magnification.

DIO, MitoLite, Hoechst
LifeGel Plates

# In Vitro Application

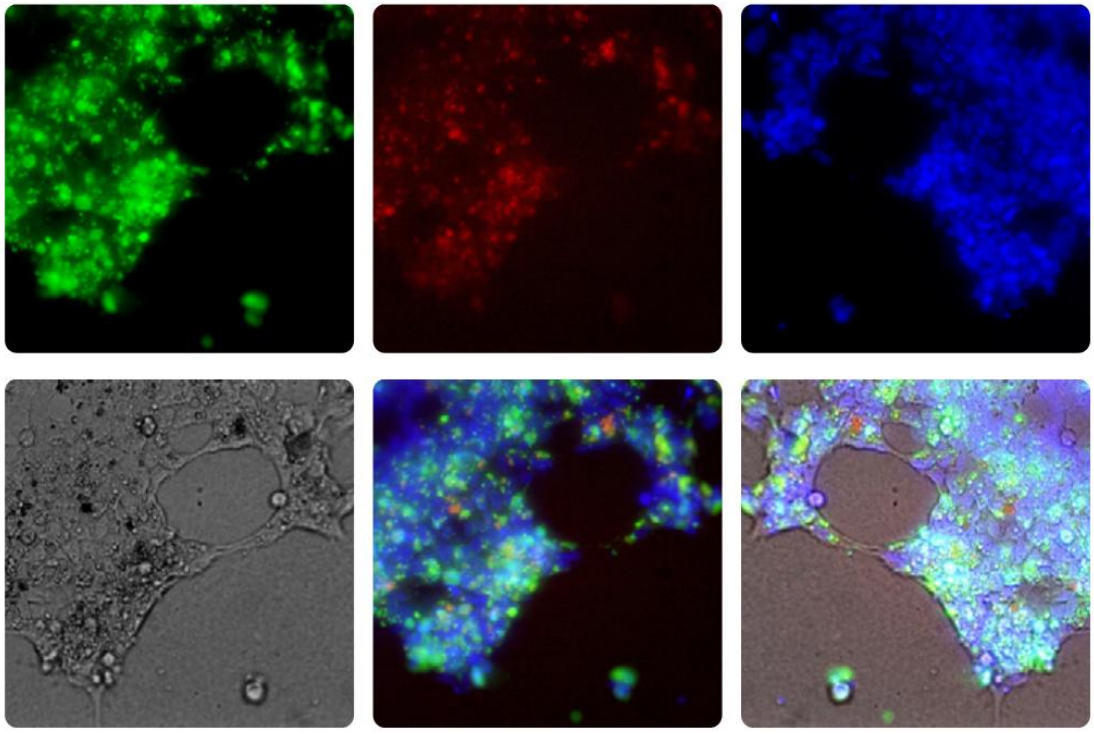

Fig.1. PANC-1 spheroid simultaneous fluorescence staining on LifeGel. Objective 10x, lens 2,5x

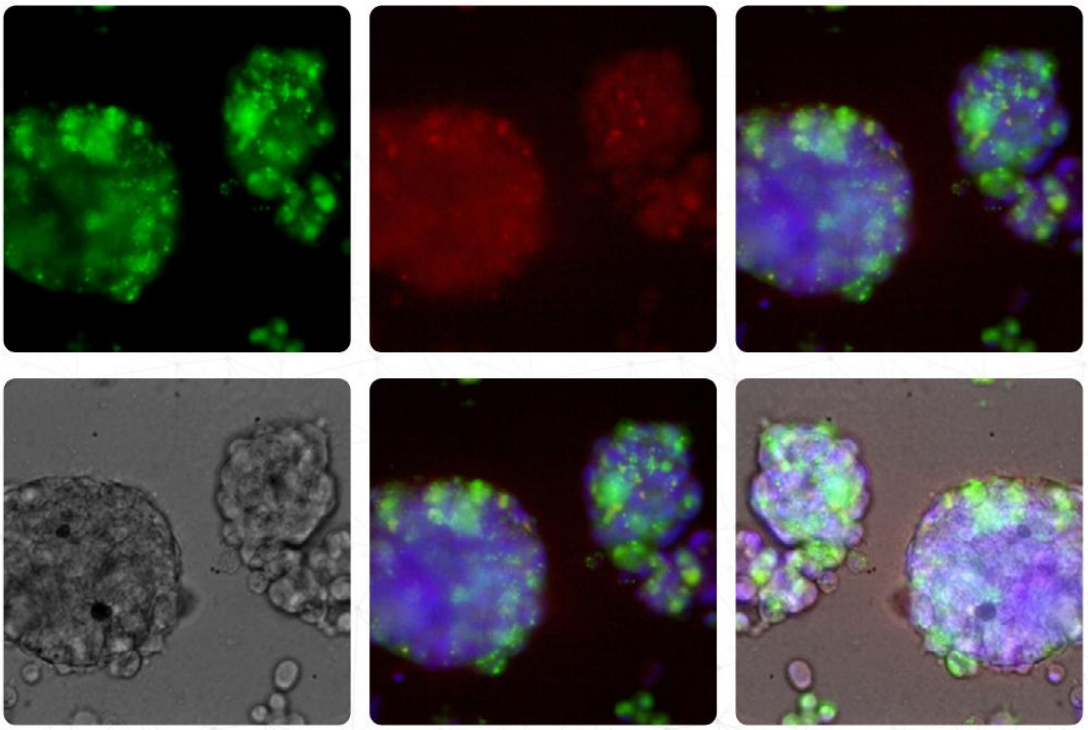

Fig.2 HEK293 spheroid simultaneous fluorescence staining on LifeGel. Objective 10x, lens 2,5x


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