Application Note

TRIPLE FLUORESCENCE STAINING - DIO, MITOLITE AND HOECHST

REAL RESEARCH

TRIPLE FLUORESCENCE STAINING - DIO, MITOLITE AND HOECHST

PROCEDURE

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.

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

On the day of the main experiment prepare working solution of MitoLite.

1. Add 20 μL of MitoLite stock solution to 10 mL of Hanks balanced salt solution (HBSS) with 20 mM HEPES (creating HHBS).
2. Before adding MitoLite working solution, supplement cells with Hoechst to obtain concentration of Hoechst 10 μg/mL.
3. Before staining, carefully wash LifeGel/cell-containing wells twice with HBSS.
4. Add 100 μL of staining solution per well (mix of Mitolite and Hoechst).
5. Incubate cells for 60 minutes at 37 ºC.
6. Wash twice with HBSS and leave in complete DMEM.
7. Take images of fluorescence and brightfied using fluorescent microscope with different magnification.

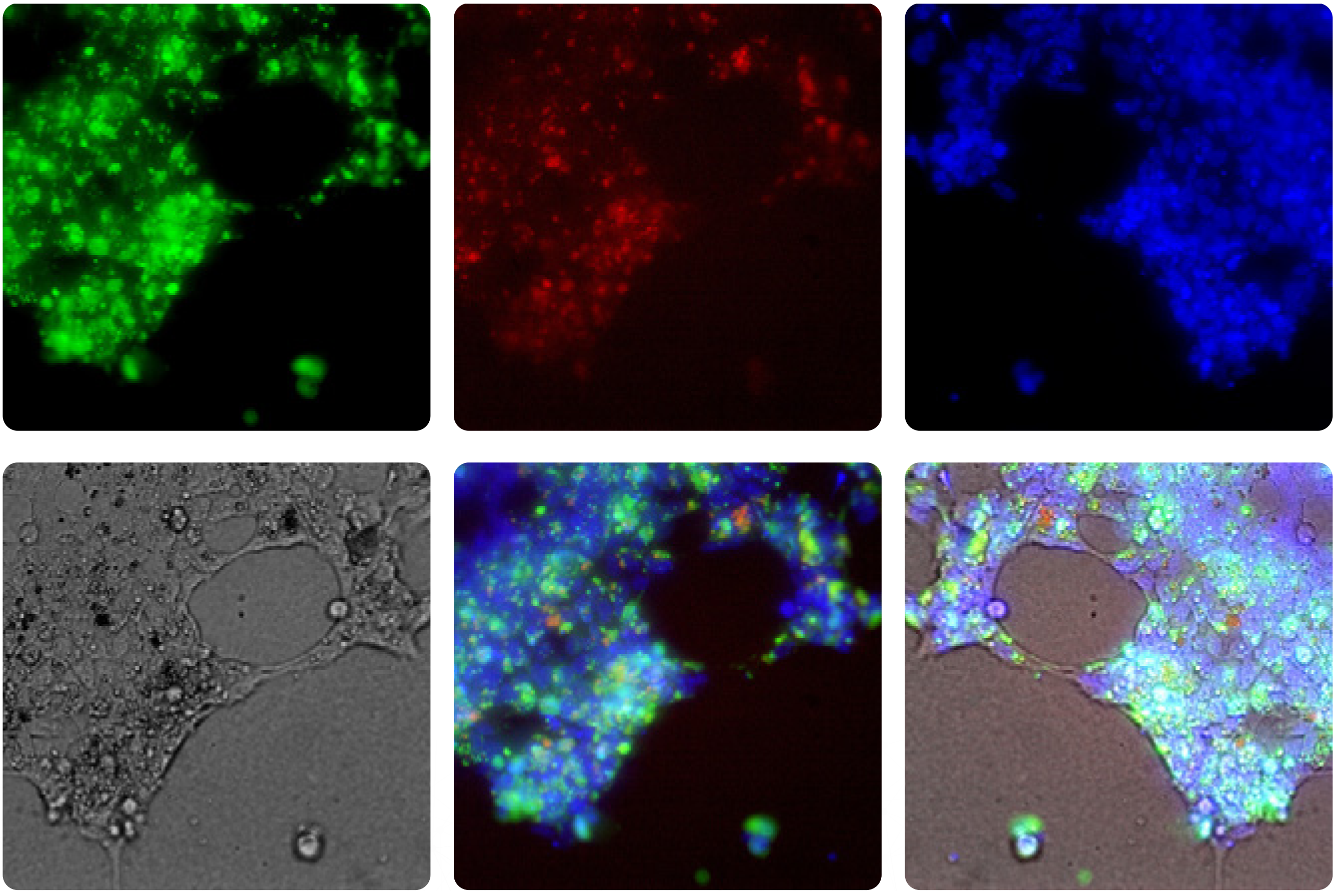

Fig.1. PANC-1 spheroid simultaneous fluorescence staining on LifeGel. Objective 10x, lens 2,5x

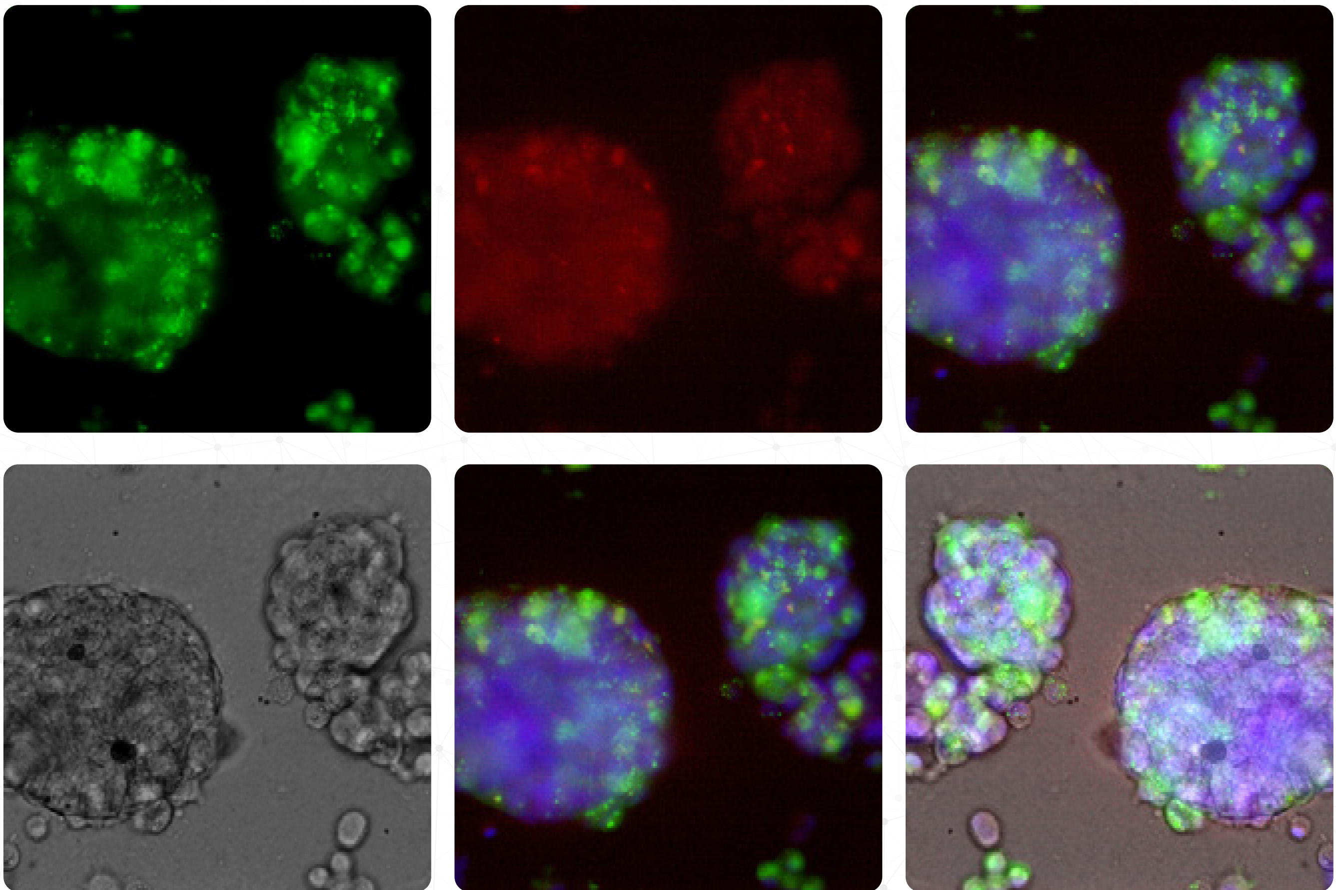

Fig.2 HEK293 spheroid simultaneous fluorescence staining on LifeGel. Objective 10x, lens 2,5x

Find out more on LifeGel and another application notes at

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