In Vitro Application

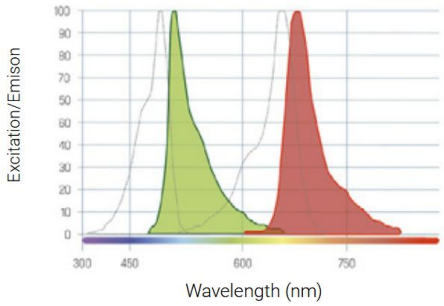
APPLICATION NOTE:
Cell Staining on LifeGel Using Vybrant™ Multicolor Cell-Labeling Kit (DiO, DiD) (Invitrogen)

DESCRIPTION

Vybrant™ consist of two lipophilic fluorescent dyes, which locate in cell phospholipid membranes.

- DiO is excited with blue light and emits green light (like GFP or FITC)
- DiD is excited with yellow/red light and emits fluorescence in far red spectrum (like Cy5, cyanine 5).

The **Vybrant™ Multicolor Cell Labeling Kit** contains 1 mL each of the DiD and DiO cell-labeling solutions. These dye delivery solutions can be added directly to normal culture media to uniformly label suspended or attached culture cells for use in cell-cell fusion, cellular adhesion and migration applications.



Days storage conditions

They should be stored at room temperature and protected from the light. Avoid freezing. Centrifuge
shortly before usage

Materials required:

- PANC-1 human pancreatic cancer cells
- Complete culture medium (e.g., DMEM + 10% FBS + antibiotics)
- PBS
- 48- or 96-well LifeGel plate
- Vybrant™ Multicolor Cell-Labeling Kit (Invitrogen)
- Pipette/multichannel pipettor

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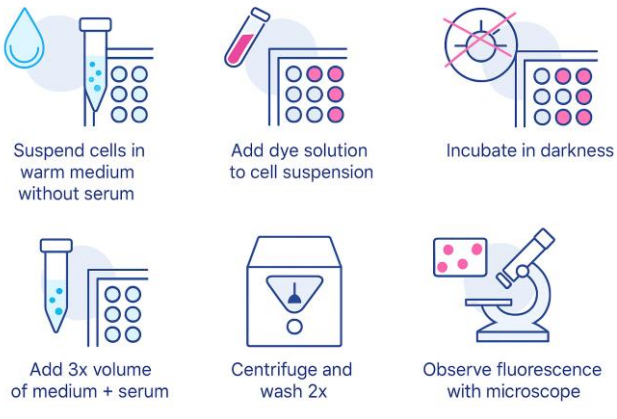
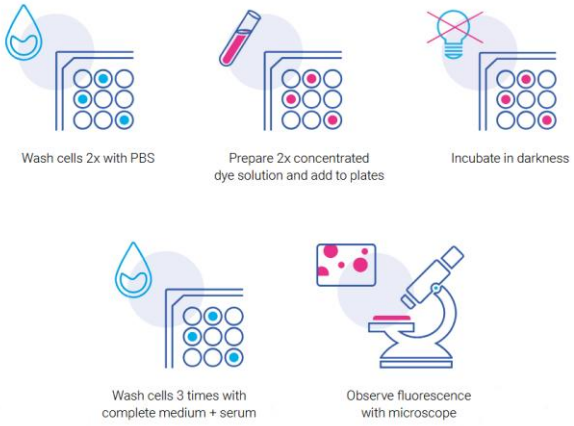
BEFORE YOU START READ OUR TIPS

- When performing staining and during work with stained cells avoid unnecessary exposure to light.
- If staining is performed for cells in suspension use 10^6 cells in 1 mL of medium without serum. If you need to stain cells in densities lower than 10^6 cells per 1 mL, then a longer incubation time is necessary. Dyes are strongly hydrophobic and will create micelles if the suspension of cells is too diluted.
- For 1 mL of cell suspension use 5 µL of the dye. If you need to maintain the fluorescence signal for a long culture time, you can try a higher concentration, but remember that too high can be toxic (50 µL/mL for PANC-1). Therefore, it may be necessary to test toxicity of higher dye concentrations for type of cells.

Option A



Option B



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PROTOCOL:

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Option A

Staining cells in suspension before seeding on LifeGel (according to manufacturer's instructions):

- Suspend 1 million cells per 1 ml of warm medium without serum.
- Add from 5 to 25 µL* dye solution per 1 mL of the cell suspension.
- Incubate in darkness for 30 minutes** at 37ºC. Mix every 5 minutes by gentle pipetting.
- After incubation stop staining by adding a 3x higher volume of serum containing medium than the initial cell suspension volume.
- Centrifuge the staining suspension cells at 1500 x g for 5 minutes.
- Remove the supernatant and gently resuspend the pellet in complete warm medium.
- Repeat the washing procedure (steps 5 and 6) two more times.
- Cells are ready for seeding on LifeGel.
- For preparing LifeGel plates and seeding check **LifeGel plates preparation for cell based assays** protocol.

  IMPORTANT: Remember to count cells again after staining, because some cells are
- always being lost during the procedure.
- After seeding you can observe cells with fluorescence microscope immediately.

** Depending on how long you need staining to be maintained: 5 µL for few days, 25µL at least two weeks.*

*** Time of incubation with dyes depends on cell type. For PANC-1 we recommend 30 minutes, however for other cells start with 15 minutes and subsequently optimize as necessary to obtain uniform labeling.*

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PROTOCOL:

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Option B
Staining cells on LifeGel:

- Wash LifeGel plates containing cells twice with PBS to remove medium containing serum.
- Prepare 2x concentrated dye solution (according to manufacturer's instruction) in warm medium without serum (50 µL/ml for strong labeling). Be careful – too high concentrations of dyes can be toxic for the cells!!!
- Add 2x concentrated dye solution: 50 ul/well for 96-well plate or 200 µL/well for 48-well
- plate and incubate in the darkness at 37° C for 30 minutes.
- Add 150 µL (96-well plate) or 600 µL (48-well plate) complete medium with serum per well to stop staining.
- Wash cells carefully with complete medium two times more being careful not to damage the LifeGel or aspirate cells.
- Observe full fluorescence signal with a microscope instrument 48 hours* after staining.

**The dyes are released from the LifeGels and then are absorbed by the cells.*

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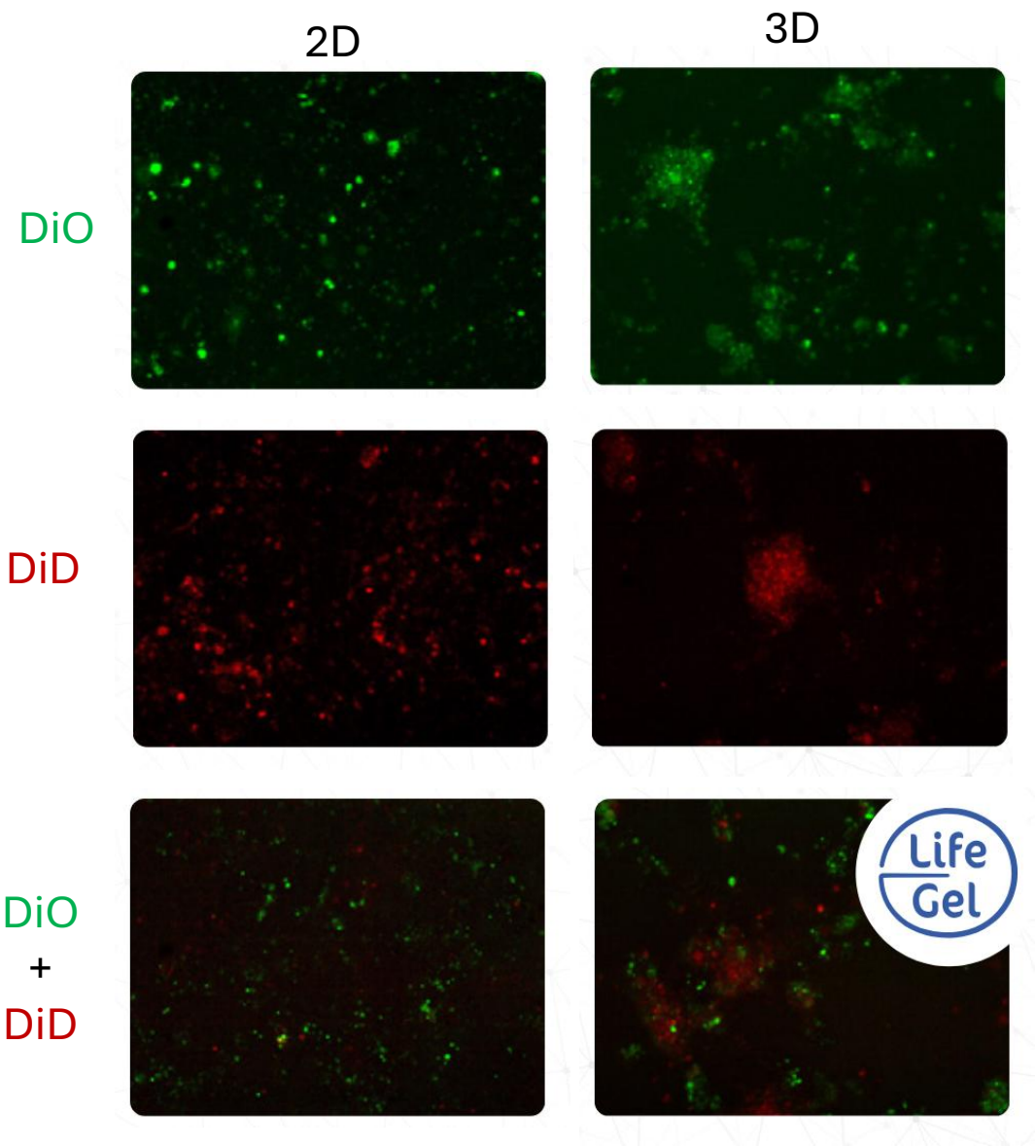

Fig. Observation of PANC-1 stained cells 7 days after seeding in 2D and 3D culture on LifeGels. Initial concentration of the cells: 3000 per well in a 48-well plate.

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