Application Note

VYBRANT™ MULTICOLOR CELL-LABELING KIT (DIO, DID)

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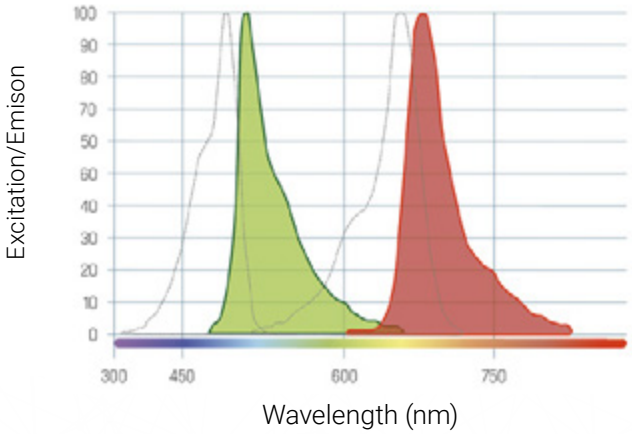
VYBRANT™ MULTICOLOR CELL-LABELING KIT (DIO, DID)

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) and DiD is excited with yellow/red light and emits fluorescence in far red spectrum (like Cy5, cyanine 5).



MATERIALS AND REAGENTS

1. LifeGel plates
2. Vybrant Multicolor Cell-Labeling Kit
3. DMEM medium
4. FBS

Days storage conditions

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

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.

EQUIPMENT

1. Laminar chamber
2. Incubator
3. Centrifuge
4. Fluorescence microscope

Option A

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

1. Suspend 1 million cells per 1 ml of warm medium without serum.
2. Add from 5 to 25 µL* dye solution per 1 mL of the cell suspension.
3. Incubate in darkness for 30 minutes** at 37ºC. Mix every 5 minutes by gentle pipetting.
4. After incubation stop staining by adding a 3x higher volume of serum containing medium than the initial cell suspension volume.
5. Centrifuge the staining suspension cells at 1500 x g for 5 minutes.
6. Remove the supernatant and gently resuspend the pellet in complete warm medium.
7. Repeat the washing procedure (steps 5 and 6) two more times.
8. Cells are ready for seeding on LifeGel.
9. Here, check how to perform 3D Cell Culture with LifeGel.

   IMPORTANT: Remember to count cells again after staining, because some cells are always being lost during the procedure.
10. 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, 25 µL at least two weeks.

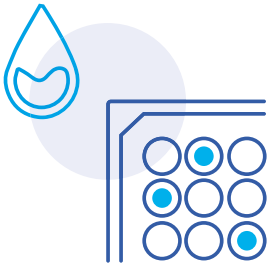
** Time of incubation with dyes depends on cell type. For PANC-1 we recommend 30 minutes, however for other cells start with 15 minutes and subsequently optimize as necessary to obtain uniform labeling.

Option B

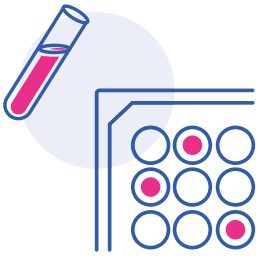
Staining cells on LifeGel:

1. Wash LifeGel plates containing cells twice with PBS to remove medium containing serum.
2. 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!!!
3. 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 37º C for 30 minutes.
4. Add 150 µL (96-well plate) or 600 µL (48-well plate) complete medium with serum per well to stop staining.
5. Wash cells carefully with complete medium two times more being careful not to damage the LifeGel or aspirate cells.
6. Observe full fluorescence signal with a microscope instrument 48 hours* after staining.

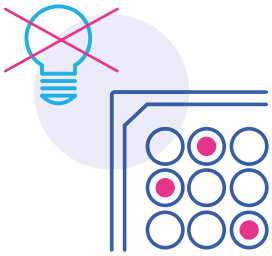
*The dyes are released from the LifeGels and then are absorbed by the cells.



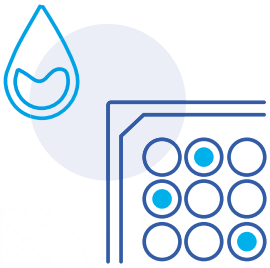
Wash cells 2x with PBS



Prepare 2x concentrated dye solution and add to plates



Incubate in darkness



Wash cells 3 times with complete medium + serum

Observe fluorescence with microscope

Fig.1. Suggested workflow prepared for option B.

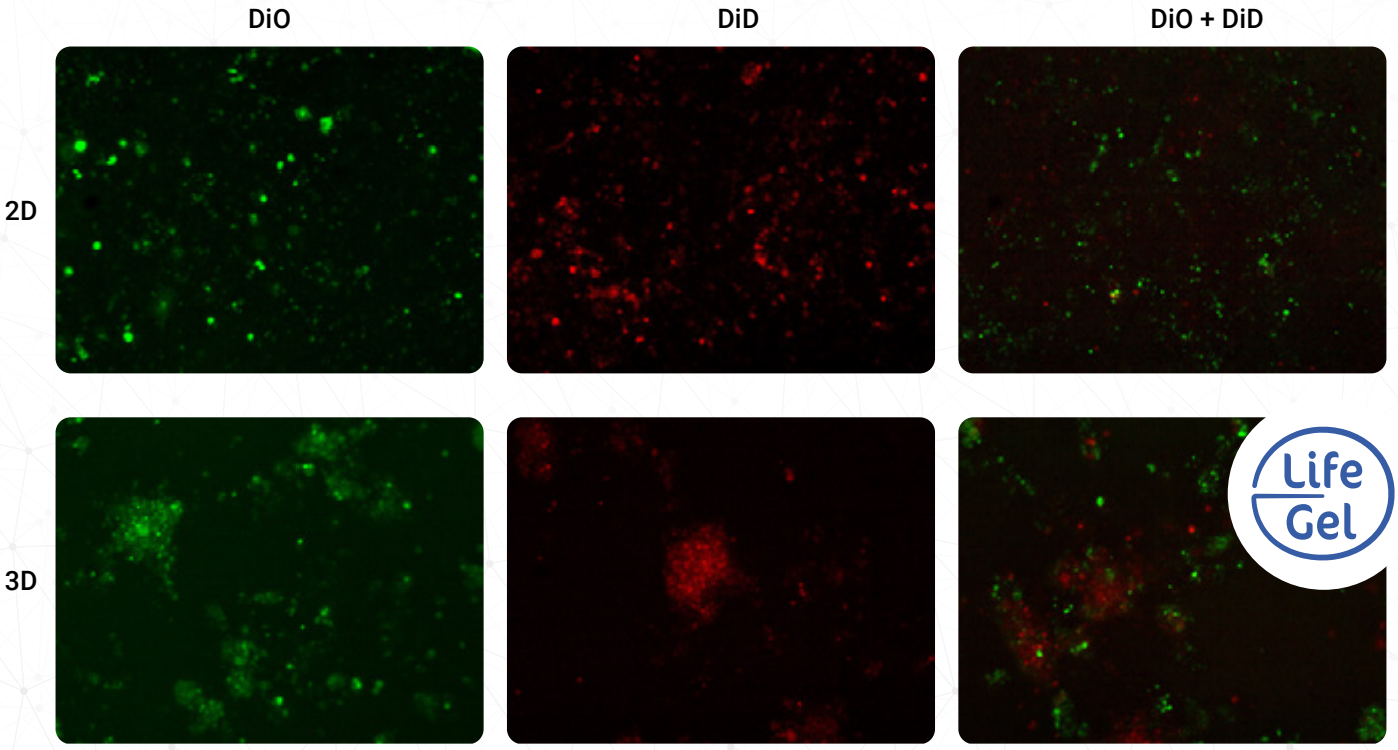


Fig 2. Observation of PANC-1 stained cells 7 days after seeding in 2D and 3D culture on LifeGels. Initial concentration of the cells: 3000 per well in a 48-well plate.

TROUBLESHOOTING

Possible problems	Proposed solutions
Observed cytotoxic effect of dyes on cells	Try to reduce the concentration of dyes
A high background	Extend the washing time procedure
Weak labeling signal	Too low cell concentration or too short incubation time with dyes

Find out more on LifeGel and another application notes at
www.real-research.com/our-science

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