Labeling Cells with Live Dyes

This protocol provides a way to stain live cells by using the following three dyes from Molecular Probes (www.probes.com); email: tech@probes.com; tel: 541-465-8300

1. CellTracker Green BODIFY (Cat # C-2102)
2. CellTracker Red CMTPX (Cat # C-34552)
3. CellTrace FarRed DDAO-SE (Cat # C-34553)

The protocol for each dye is no different from each other, however, due to the filter we have and possible dyes quality, I found the intensity for same amount of dye,
Green _ Red __ Far Red
I would suggest use 5 to 10 times more amount of Far Red when applying the following protocol.

The amount of dye used in the protocol is good in live cells for 4+ days.

Preparation:
One first needs to make stock solution (10mM) for each dye. I have already aliquot Green dye in 20 0.5mL eppendorf tubes because the Green dye we ordered came in as one big vial (1mg), while red and far red came in as 20 different vials (50ug each). So people who need to use red or far red will have to aliquot one of the vial.
Use pure DMSO to dissolve lyophilized dye.
The amount of DMSO for each vial(50ug) to be used is calculated and listed as follows:

For Red: 7.3 uL
For FarRed: 9.9 uL

Both aliquots and lyophilized dye are stored in -20 in a big zip-loc bag. Green dye are in 20 different aliquots already and can be found inside a smaller yellow-green zip-loc bag within the big zip-loc bag.

Protocol (for the embedded beads with PAE + embedded T47D in gel)

1. Grow cells on plates to desired confluence (it depends on when you are going to use the stained cells and how fast the cells grow, for instance, if I want to use the stained tumor cells the 2nd day, I would stain them at around 50% confluence the day before so at the 2nd day they will be at least 80~90%)
2. I use 5uM of dye for this experiment. (It is recommended in the protocol, for long-term staining or rapid dividing cells, use 5-25uM dye. 0.5-5uM for shorter experiment.) Use serum-free media to dilute the dye to the desired concentration.
3. When the cells have reached the desired confluence, remove the medium from the dish and add the pre-warmed dye for 15-45 minutes at 37_.
4. Replace the working solution with culture medium and incubate for another 30 minutes at 37_.
5. The stained cells are now ready to be used for the follow-up experiment.

There is a flowchart type of protocol I actually used when I've stained the cells as a reference.

Flowchart to stain live cells:

Dye stock solution (10 mM)
9.9uL for Far Red
7.3uL for red

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Dilute with serum-free medium (5uM)
1.5 uL stock in 3mL serum-free medium (for 60mm plates)

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Stain cells for 45 minutes @ 37_
(cells are 50% confluence)

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Replace with cell culture medium @ 37_

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Cells were used in the follow-up beads experiment next day. However, it is certainly cells after the last step can be used right away and I've tried that too and the intensity doesn't show much a difference.

Note:
1. For the beads experiment, I used 2 well chamber slides, basically followed the same protocol Claudia has, except following recipe of the gel.

Gel:
10mg/mL fibrinogen…….. .250 uL
Collagen (vitrogen)……..250 uL
Beads/stained PAE……...250 uL
Stained tumor cells (>90% confluence, resuspend in PAE media).....250 uL
CaCl2 (2mg/mL)…….100 uL
Thrombin…….....20 uL

2. Molecular probes also gave the manual together with protocol with the shipment. I asked Claudia if the lab keep these manuals or protocols in a folder and she said yes and she would take care of it. So the lab should have manual too. However, if somehow nobody can find it, I have a copy of it too (with my messy writings on top of them). If needed, I will stop by and give them. =)

3. Good Luck!!