**Procedure for live/dead cell analysis with cell tracker**
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**Supplies**

- PBS or HBSS
- FBS free media
- Propidium Iodide PI
    - Invitrogen # P3566
- CellTracker Green CMFDA
    - Invitrogen #C7025 – special packaging.

**Notes:**

- These are stains for non-fixed/ live cells. By adversely handling cells in a manner wherein they might die prior to fluorescent imaging will lead to false positives with regard to PI staining.
    - Examples of adverse cell conditions
        - Not maintain cells in fluid environment
        - Maintaining cells in PBS or HBSS for longer than 30 minutes at room temperature and longer than 1 hour at 37 degrees C.
        - Maintaining at room temp longer than 30 minutes
- PI stains almost instantaneous. Any residual PI in the cell solution can cause newly dead cells to stain red, altering time point estimate.

**Solution Preparation:**

1. Warm PI solution and 1 50-microgram vial of cell tracker to room temperature.
2. Dissolved cell tracker to a final concentration of 10mM with DMSO
    a. 10.8 microliters of DMSO per 50 microgram aliquot
3. Dilute in FBS free media to a concentration between 0.5 and 25 microM
    a. 5 microM: Dilute total volume (10.8 microL) into 21.6 mL of FBS free media
    b. **10 microM: 10.8 into 10.8 mL**
    c. 15 microM: 10.8 into 7.2 mL
    d. 20 microM: 10.8 into 5.4
4. PI solution can be diluted from Invitrogen provided stock.
5. Maintain cell tracker solution in 37 degree water bath, not completely exposed to ambient light. If in hood, with light on, keep in Al foil. PI solution should be kept in amber plastic bottle provided by manufacturer until time of use, stored in refrigerator if possible.

**Example Experimental Conditions for Transwell Filters:**

1. Add 250 microliters of cell tracker solution to each well.
    a. Cell Tracker can be in contact with cells without harming them for 48 hours or more.
2. Add 0.5 microliters of 1 mg/mL PI solution to two of the wells.
3. Incubate for 30 minutes in incubator.
4. Add 0.5 microliters of 1 mg/mL PI solution to two additional wells.
5. Aspirate off cell staining solution of prior PI stained wells (in step 2) and add 500 microliters of PBS or HBSS. Aspirate off.
6. With a scalpel and tweezers, cut the filter from the holder and place on a glass slide, face side up.
7. With a pipet (transfer or otherwise) drop at least 100 microliters of PBS or HBSS onto filter surface.
8. Sandwich with glass slide or coverslip.
9. Image.
10. Go to step 4 and repeat until all samples are done.

- Live cells should show strong FITC (green) staining due to staining of cellular membrane.
- Dead cells will show both membrane stains. The PI (red) stain should be a nuclear stain.