SOP TITLE:	VERSION:	
PM-PD-SOP3 LIVE/DEAD Stain for Cell Viability	1.2	
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1. Background

The LIVE/DEAD kit is a two-color fluorescence cell viability assay. Calcein AM, which is enzymatically converted to the highly fluorescent calcein by intracellular esterase activity, can be used to detect live cells since it is cell-permanent and well retained within live cells. Ethidium homodimer, which is excluded by the intact plasma membrane of live cells, enters cells with damaged membranes and undergoes a 40-fold enhancement of fluorescence on binding to nucleic acids, producing a bright red fluorescence in dead cells.

2. Materials

- Class II Microbiological Safety Cabinet
- Cells for staining
- Parafilm
- Scalpel blade (for hydrogels)
- Glass coverslips or imaging dishes (for hydrogels)
- P1000, P10 filter tips
- P1000, P10 pipette
- DPBS
- Foil
- Universal tubes/Eppendorfs
- Fluorescence microscope

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Product	Supplier	Cat No	Storage
DPBS	Gibco	14190-094	RT

LIVE/DEAD kit	Fisher	L3224	-20 °C

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3. METHODS

Staining Cells in Hydrogels

1. Thoroughly defrost the staining reagents.
2. Prepare staining solution (2 µL/mL Ethidium homodimer, 0.5 µL/mL calcein AM) in PBS. Ensure it is well mixed. Cover in foil to protect from the light.
3. Wash gels with PBS.
4. Cover the gels with staining solution and incubate in the dark for 10-15 minutes.
5. Remove excess staining solution.
6. Read signal on plate reader and/or visualise under a confocal microscope.

Staining gels in a cell culture insert

The same basic procedure as above can be used, but you may need to first remove the gels from the cell culture insert and mount them on a coverslip prior to staining.

Imaging

For imaging of live cells, the calcein AM has an excitation/emission of 494/517 nm. For imaging dead cells, the ethidium homodimer-1 has an excitation/emission of 528/617 nm.

4. Disposal

1. Dispose of cell culture medium, and any solutions that have been in contact with cells, by pouring into waste pot/ aspirating into vacuum trap containing Chemgene. Leave in Chemgene for at least one hour.
2. Dispose of any unneeded glass in appropriate glass bins.
3. Dispose of contaminated sharps in appropriate sharps bins.

User Initials Legend

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