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LIVE/DEAD Cell Viability Protocol

Assay Description

Live cells are distinguished by the presence of ubiquitous intracellular esterase activity, determined by the enzymatic conversion of the virtually nonfluorescent cell-permeant calcein AM to the intensely fluorescent calcein. The polyanionic dye calcein is well retained within live cells, producing an intense uniform green fluorescence in live cells (ex/em ~495 nm/~515 nm). EthD-1 enters cells with damaged membranes and undergoes a 40-fold enhancement of fluorescence upon binding to nucleic acids, thereby producing a bright red fluorescence in dead cells (ex/em ~495 nm/~635 nm). EthD-1 is excluded by the intact plasma membrane of live cells. The determination of cell viability depends on these physical and biochemical properties of cells. Cytotoxic events that do not affect these cell properties may not be accurately assessed using this method. Background fluorescence levels are inherently low with this assay technique because the dyes are virtually non-fluorescent before interacting with cells.

Here we are using the LIVE/DEAD assay to assess the viability of cells cultured in PureCol® collagen hydrogels using combination reagents of calcein AM and EthD-1. The calcein will bind to live cells and produce green fluorescence while EthD-1 will bind to dead cells and show red fluorescence.

Materials Needed

- PureCol® (Cat #5005)
- Cells/cell lines of interest
- Cell culture media
- LIVE/DEAD ® Viability/Cytotoxicity Kit *for mammalian cells* (Invitrogen, Cat #L3224)
- Cell culture plates
- 15 ml or 50 ml centrifuge tubes
- Serological pipettes
- Micropipette and tips
- D-PBS
- Fluorescence microscope

LIVE/DEAD Cell Viability Kit Contents:

Component A: Calcein AM, two vials, 40 µL each, 4 mM in anhydrous DMSO
Component B: Ethidium homodimer-, two vials, 200 µL each, 2 mM in DMSO/H2O 1:4 (v/v)

Storage and Handling of Reagents:

Reagents in this kit should be stored sealed, desiccated, protected from light and frozen at ≤ -20°C. Allow the reagents to warm to room temperature and centrifuge them briefly before opening. Before refreezing, seal all stock solutions tightly. Calcein AM is susceptible to hydrolysis when exposed to moisture. Prepare aqueous working solutions containing calcein AM immediately prior to use and use within one day. EthD-1 is stable and insensitive to moisture. Stock solutions of EthD-1 in DMSO/ H_2O or other aqueous media can be stored frozen at ≤ -20 °C for at least one year.

Procedures

1. Culture cells in PureCol® hydrogel following the Hydrogel Cell Culture Protocol.

2. Remove the LIVE/DEAD® reagent stock solutions from the freezer and allow them to warm to room temperature.

3. Add 20 µL of the supplied 2 mM EthD-1 stock solution (Component B) to 10 mL

of sterile D-PBS and vortex briefly to ensure thorough mixing. This gives an approximately 4 µM EthD-1 solution.

4. Combine the reagents by transferring 5 µL of the supplied 4 mM calcein AM stock solution (Component A) to the 10 mL EthD-1 solution. Vortex the working solution to ensure complete homogenization.

5. Aspirate the cell culture media in the wells plated with collagen hydrogels and rinse the hydrogel briefly with D-PBS.

6. Add the 2 µM calcein AM and 4 µM EthD-1 working solution directly to cell-cultured collagen hydrogels.

   Note 1: Depending on the size of hydrogel/well plate, the volume of the working need to be adjusted and ensured to cover the entire hydrogel. Recommended volumes are listed in Table 1:

Table 1. Working solution volume per well

Plate size	Volume
6 well	1.5 ml
12 well	1 ml
24 well	0.5 ml
48 well	0.3 ml
96 well	0.1 to 0.2 ml

   Note 2: The aqueous calcein AM solution is susceptible to hydrolysis so it should therefore be used within one day.

7. Cover the plate with aluminum foil and incubate the cells/hydrogels for 30 to 45 minutes at room temperature. A shorter incubation time may be used if the dye concentrations or incubation temperature are increased.

8. After incubation, remove the staining solution and rinse the hydrogel with D-PBS three times. Add D-PBS to the hydrogel and proceed to imaging.

9. Transfer the plate to a fluorescence microscope and view the labeled cells. Select GFP channel for imaging live cells and RFP channel for dead cells.

10. Save images from the two channels as separate .tif files for further analysis. Images can be merged in ImageJ software for presentation.

Notes

1. This procedure is used for making a 10ml solution of combined reagents. To save reagents, a specific volume of solution can be calculated based on the number of testing samples.

2. The reagents are susceptible to direct light so make sure to turn off lights when handling the reagent vials and conducting experiments. Also, wrap reagent vials with aluminum foil for storage.

3. The samples that have already been dyed cannot be reused. Please prepare sufficient samples to run the assay at different time points.

Note: This protocol can work with a broad variety of hydrogels. Please view our entire portfolio to find the right hydrogel for your cells, or contact a technical support representative for assistance.

