**Live-Dead Cytotoxicity Assay Kit, for mammalian cells MCT8010**

****

**Technical literature is available at:** [**www.mesgenbio.com**](http://www.mesgenbio.com)**. E-mail MesGen Technical Services if you have questions on use of this system: tech@mesgenbio.com**

**Introduction**

Distinguishing between live and dead cells is very important for investigation of growth control and cell death. The Live-Dead Cytotoxicity Kit provides the ready-to-use reagents for convenient discrimination between live and dead cells. The kit utilizes Calcein,AM, a cell-permeable green fluorescent dye (Ex/Em = 488/518 nm), to stain live cells. Dead cells can be easily stained by propidium iodide (PI), a cell non-permeable red fluorescent dye (Ex/Em = 535/615). Stained live and dead cells can be visualized by fluorescence microscopy using a band-pass filter.

**Kit Components**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **100T** | **500T** | **1000T** |
| Solution A (1 mM Calcein,AM)  | 50l | 250l | 500l |
| Solution B (1 mg/ml PI)  | 50l | 250l | 500l |
| Staining Buffer | 50ml | 250ml | 500ml |

**Cell Staining Protocol**

1. Prepare enough Staining Solution for your assay (0.5 ml per well in 24 well dish) : mix 1l of Solution A and 1l of Solution B in 1 ml of Staining Buffer. Scale up accordingly for larger numbers of assays.

2. Collect cells (1 x 106cells) by centrifugation at 500 X g for 5 min.

3. Resuspend to 0.5 ml Staining Solution

4. Incubate for 15 min at 37°C.

5. Place the cell suspension on a glass slide. Cover the cells with a glass coverslip. For analyzing adherent cells, grow cells directly on a coverslip. Following incubation with the Staining Solution, invert coverslip on a glass slide and visualize cells.

6. Observe cells immediately under a fluorescence microscope using a band-pass filter. Healthy cells stain only the cell-permeable Calcein,AM, fluorescing green. Dead cells can stain both the cell-permeable Calcein,AM and the cell non-permeable PI (red), the overlay of green and red appears to be yellow-red.

**Caution**

As the optimal staining conditions may vary among different cell types, we recommend that a suitable concentration of Solution A and B be determined individually. Please note that PI is suspected to be highly carcinogenic, so careful handling of the reagent is required.

**Storage**

Store at-20°C. Protect from light.

***For Research Use Only. Not For Use In Diagnostic Procedures.***