



## CFDA/EtBr Dual Stain Viability Assay

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Cell viability data is often used for the interpretation of *in vitro* genotoxicity assay results. However, standard methods often used (e.g., trypan blue exclusion; mitotic index) are limited to measuring cell membrane permeability or cell division which are not always indicative of cell death and which can be affected by technical methods (e.g., cell scraping) and/or test compound mechanisms (e.g., cell cycle arrest) unrelated to cell death. Therefore, the determination of the metabolic competency of exposed cells may be a better viability measurement to complement DNA damage assays. The CFDA/EtBr dual stain viability assay allows for the determination of both plasma membrane integrity as well as the metabolic competency of the cells. Once stained, metabolically active cells cleave CFDA in the cytoplasm resulting in green fluorescence. Meanwhile, the ethidium bromide will intercalate the DNA in the nucleus of cells with a permeable plasma membrane resulting in a red fluorescence. Samples are scored by counting 100 cells each for the number of green cells, red cells, and the number of cells that are both red and green (red/green). Cells that fluoresce green are categorized as viable. Red/green are considered compromised but metabolically active and are therefore categorized as viable. And cells that fluoresce red in the absence of green fluorescence are categorized as dead.

### Dual Stain Preparation (20 mL final volume)

1. 5(6)-Carboxyfluorescein (CFDA; CAS 124387-19-5) Stock Solution (10 mL final volume)
  - a. Dissolve 0.003 g of CFDA in 1.0 mL acetone.
  - b. Add 420  $\mu$ l of this stock to 9.58 mL PBS.
2. Ethidium Bromide (EtBr; CAS 1239-45-8) Stock Solution (10 mL final volume)
  - a. Add 0.05 g EtBr to 1.0 mL 100% ethanol\* and mix.
  - b. Add 100  $\mu$ l of this stock to 4.9 mL PBS.
  - c. Add 250  $\mu$ l of the second mixture to 9.75 mL PBS.
3. Mix the CFDA stock solution and EtBr stock solution 1:1.
4. Store in the dark at 1-10 °C for up to 6 months.

**\*NOTE:** Ethanol with any trace of methanol added will cause stain induced cell death. **ONLY** 100% (200 proof) Ethanol can be used for this stain.

### Dual Stain Viability Assay

1. To every 50  $\mu$ l of cell suspension, add 50  $\mu$ l of the dual stain solution and mix.
2. Incubate at 37 $\pm$ 3 °C for 3-5 minutes.
3. Add 500-1000  $\mu$ l buffered saline solution and mix.
4. Centrifuge at 1-10°C and remove supernatant.
5. Repeat steps 3 and 4 leaving ~50  $\mu$ l of the supernatant with the cell pellet.
6. Cell samples should now be maintained on ice.
7. Immediately prior to scoring each sample\*\*, resuspend the cell pellet in the remaining supernatant and put the suspension on a clear microscope slide.

**\*\* Note:** It is critical to freshly prepare each sample to a slide immediately before scoring to avoid continued cellular activities or drying of the sample that could occur at room temperature and that may affect viability analysis results.

8. Cover the cell suspension sample on the slide with a 22 x 22 mm coverslip and view the cells with a fluorescent microscope using a FITC long band pass filter combination (Acridine Orange filter).
9. Count 100 cells per sample assigning the cells to one of the following three categories:

<sup>a</sup>Green (viable)

<sup>b</sup>Red and green (compromised but viable)

<sup>c</sup>Red (dead)

