Fluorescence Cell LIVE/DEAD Viability Assay

References

1. Nicholson, A. M., Wold, L. A., Walsh, D. M., & Ferreira, A. (2012). β-Amyloid carrying the Dutch mutation has diverse effects on calpain-mediated toxicity in hippocampal neurons. *Molecular medicine (Cambridge, Mass.)*, *18*(1), 178–185. https://doi.org/10.2119/molmed.2011.00366
2. Rapoport, M., Dawson, H. N., Binder, L. I., Vitek, M. P., & Ferreira, A. (2002). Tau is essential to beta -amyloid-induced neurotoxicity. *Proceedings of the National Academy of Sciences of the United States of America*, *99*(9), 6364–6369. https://doi.org/10.1073/pnas.092136199

Kit

1. LIVE/DEAD™ Viability/Cytotoxicity Kit, for mammalian cells, Invitrogen, Cat# L3224

Abbreviation

1. PBS: Phosphate Buffered Saline

Protocol

1. Allow the LIVE-DEAD Determination kit to warm up to room temperature.
2. Wash coverslips containing hippocampal neurons with sterile PBS at room temperature.
3. Prepare 2.5 ml of a reagent solution containing 4 µM EthD-1 and 2 µM calcein in PBS per 35 mm dish. Vortex.
4. Incubate coverslips in the solution for 20 min at 37°C.
5. Rinse coverslips with PBS.
6. Mount coverslips on clean glass slides without mounting medium.
7. Seal coverslips with nail polish.
8. View the labeled cells under the fluorescence microscope immediately. Count live and dead cells per field x 10 fields from 3 different culture preparations and calculate the ratio live/dead cells per experimental condition.