

ACTIVTox cyp1A Induction Assay Protocol

Description

Cyp1A is a member of the P450 family of enzymes in the liver. It is induced by a variety of agents such as cigarette smoke, omeprazole, methylcholanthrene, and TCDD; it is inhibited by furafylline. One of the cyp1A substrates is ethox

5. Aspirate the media off the cell plate(s) taking care not to touch the cell layer. If using multiple cell plates, limit the number handled at one time so that the cells are without media for no more than 5 minutes.
6. Pipet 100 μ L of the test compounds/ MED #7 solution and the positive and negative controls in quadruplicate into the wells of the cell plate(s). (See the recommended cell plate layout.)
7. Incubate the cell plate(s) for 24 hours in a 37°C \pm 2°C, 5% \pm 1% CO₂, 90% \pm 5% humidity incubator.
8. Aspirate the liquid from all wells of the cell plate.
9. Pipet 100 μ L of the 10 μ M ERes/MED #6 solution into each well of the cell plate(s).
10. Incubate the cell plate(s) for 1 hour in a 37°C \pm 2°C, 5% \pm 1% CO₂, 90% \pm 5% humidity incubator.
11. Measure the fluorescence of each well at an excitation wavelength of 530 \pm 20 nm and an emission wavelength of 590 \pm 20nm.

Recommended Plate Layout

