

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 ethoxyresorufin (ERes) which, in the presence of cyp1A, is converted to the fluorescent compound resorufin. This specific reaction makes ERes an excellent probe for measuring cyp1A activity.

The ACTIVTox kit provides the user with the ability to study cyp1A induction in response to compounds in a very rapid, robust and reproducible manner. The assay entails a 24 hour compound treatment followed by a 1 hour substrate reaction. Fluorescence intensity is proportional to the activity of the cyp1A enzyme.

Upon Receipt of Cells

Upon receiving the cell plate(s), inspect and feed the cells as stated below:

1. Cell plate preparation must be performed in a sterile environment using standard tissue culture techniques.
2. Remove and discard the plastic lid from the cell plate.
3. Inspect the cell plate for
 - a. peeling or detachment of cells in the wells.
 - b. damage to the plastic sealing membrane.
 - c. loss of media from the well(s).

If any of these conditions exist, discard the cell plate and Call Stem Cell Innovations Customer Service at 281-679-7900 for a replacement.

4. Remove the plastic seal by carefully lifting the edges of the seal and pulling gently across the width of the cell plate. Discard the plastic seal.
5. Aspirate the media from the wells of the cell plate. Discard the media as biological waste.
 - a. Multichannel pipets or aspirators should be used.
 - b. Take caution not to disturb the cell layer.
6. Pipet 200µl of fresh, room temperature Med #7 into each into each well.
7. Place a new sterile lid on the cell plate.
8. Incubate the cell plate overnight in a 37°C ± 2°C, 5% ± 1% CO₂, 90% ± 5% humidity incubator.

9. The *cyp1A* Induction Assay can be started the next day after this initial feeding.

Materials Required for the *cyp1A* Assay

1. Cell plate(s) with C3A cells.
2. Ethoxyresorufin in DMSO (ERes), 1mM. Store at 2 – 8 °C until needed for assay.
3. Media #6 (MED #6). Store at 2 – 8 °C until needed for assay.
4. Positive control – Methylcholanthrene, 100X, (500 µM). Store at 2 – 8 °C until needed for assay.
5. Media #7 (MED #7). Store at 2 – 8 °C until needed for assay.
6. Fluorescent Plate Reader (supplied by user)

Preparing the 10µM Solution of Ethoxyresorufin

NOTE: Prepare this solution prior to the development of the assay.

1. Prepare a 10µM solution of Ethoxyresorufin in MED #6 from the 1mM stock solution of Ethoxyresorufin.
2. Mix thoroughly.
3. This solution should be used within 4 hours of preparation.

Performing the *cyp1A* Assay

NOTE: All solutions required to perform this assay should be warmed to room temperature before using.

1. Determine that the cell plate(s) were prefed with MED #7 the day before starting the *cyp1A* induction assay.
2. Prepare the desired test compounds in MED #7 at the desired concentrations.
3. Prepare the positive control by diluting to 1X with MED #7.
4. Prepare the negative control by diluting your vehicle to the desired concentration in MED #7. (Note: DMSO concentration can be up to 1% final volume without any physiological effect on the C3A cells.)

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 100µL 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 ± 2°C, 5% ± 1% CO₂, 90% ± 5% humidity incubator.
8. Aspirate the liquid from all wells of the cell plate.
9. Pipet 100µL of the 10µM 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 ± 2°C, 5% ± 1% CO₂, 90% ± 5% humidity incubator.
11. Measure the fluorescence of each well at an excitation wavelength of 530 ± 20 nm and an emission wavelength of 590 ± 20nm.

Recommended Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A		Negative Control	Compound #1	Compound #2	Compound #3	Compound #4	Compound #5	Compound #6	Compound #7	Compound #8	Compound #9	
B												
C												
D												
E		Positive Control	Compound #10	Compound #11	Compound #12	Compound #13	Compound #14	Compound #15	Compound #16	Compound #17	Compound #18	
F												
G												
H												

ACTIVTox CYP1A Assay

