



ACTIVTox Cytotoxicity Assay Protocol

Description

The ACTIVTox cytotoxicity assay provides a rapid, high throughput method for quantitatively measuring lactate dehydrogenase (LDH) release from lysed cells in a colorimetric, non-radioactive system. The ACTIVTox cell line provides the user with a platform that emulates an adult liver system complete with physiologically regulated drug disposal enzymes.

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 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 cytotoxicity assay can be started the next day after this initial feeding.



Materials Required for Cytotoxicity Assay

1. Cell Plate(s) with C3A cells.
2. Cytotoxicity Assay Kit. Kit includes:
 - a. 10X Lysis Solution. Store at 2 – 8°C until needed for the assay.
 - b. Reconstituted Substrate Solution. Light sensitive. Store in a dark area until just before use. Store at –20°C until needed for the assay.
 - c. Stop solution. Store at 2 – 8°C until needed for the assay.
3. Positive Control: 100X Terfenadine (5mM). Store at –20°C until needed for the assay.
4. Media #7 (MED #7). Store at 2 – 8°C until needed.
5. Microplate Plate Reader (supplied by user)
6. ELISA Plate (supplied by user) Suggested brand – Greiner No. 655090

Performing the Cytotoxicity 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 cytotoxicity assay.
2. Prepare the desired test compounds in MED #7 at the desired concentration.
3. Prepare the negative control by using MED #7 plus appropriate amount of vehicle. (Note: DMSO concentration can be up to 1% final volume without any physiological effect on the C3A cells.)
4. Prepare the positive control by diluting to 1X with MED #7. (Note: Terfenadine can be slightly insoluble and may require heating to 37°C and vortexing before diluting.)
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 for 48 hours in a 37°C ± 2°C, 5% ± 1% CO₂, 90% ± 5% humidity incubator.
8. Approximately 45 minutes before the cell plate(s) are developed, add 10 µL of the Lysis Solution (10x) per 100µL of MED # 7 to all of the lysis control wells in the cell plate(s).
9. Gently tap on the side of the cell plate several times in order to get an even distribution of the LDH enzyme throughout the medium.
10. Pipet 15µL of deionized water into each well of an empty ELISA plate.
11. Pipet 15µL from each well of the cell plate into the designated wells of the water containing ELISA plate.
12. Pipet 30µL of the prepared substrate mixture into each well of the ELISA plate.
13. Incubate the ELISA plate in a darkened area at room temperature (25°C) for 10 minutes. (Note: Slightly shorter or longer incubation times may be required if the room is warmer or cooler respectively).
14. Pipet 30µL of the stop solution into each well of the ELISA plate.
15. Read the absorbance at 490 nm.

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	Lysed Control	Positive Control	Compound #10	Compound #11	Compound #12	Compound #13	Compound #14	Compound #15	Compound #16	Compound #17	Compound #18	
F												
G												
H												

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