

Cytosensor Microphysiometer Bioassay

Theory: The purpose of this assay is to evaluate the potential toxicity of a test article as measured by the test article induced reduction in the acidification rate in cultured cells (L929, Normal human epidermal Keratinocytes, MDCK, etc.) as measured by the cytosensor.

Experimental Procedure

Target Cell Preparation

- Stock cell flasks are incubated at $37 \pm 1^\circ\text{C}$ in a humidified atmosphere containing $5 \pm 1\%$ CO_2 in air.
- A stock cell flask is trypsinized (according to requirements of specific cell line) and suspended in the appropriate cell medium with 1% fetal bovine serum or in serum free medium (keratinocytes).
- A count of the cell suspension is made and the appropriate dilution made in the cell medium with 1% serum (or serum free medium for keratinocytes) to attain the desired cell concentration. One and one half ml of the appropriate medium are added under the membrane cups.
- One half ml of the cell suspension are added into membrane cups.
- The membrane cups are incubated for 16 to 36 hours at $37 \pm 1^\circ\text{C}$ in a humidified atmosphere containing $5 \pm 1\%$ CO_2 in air prior to use.

Assay Procedure

- 16 to 36 hours after seeding cells, the medium is removed from inside and under the membrane cups and replaced with experiment medium.
- A spacer is placed at the bottom of the membrane cup.
- An inner membrane cup (capsule insert) may be placed within the membrane cup.
- A cell capsule is placed into a sensor chamber.
- Each sensor chamber is placed onto the cytosensor.
- The experimental program (which regulates the pump flow and determines the acidification rate) is initiated and the cells are equilibrated in the experimental medium (without serum, without sodium bicarbonate, without HEPES) in the stop/flow environment.
- Once the cells are equilibrated in the system, serial dilutions of the test article (starting at the lowest concentration) are introduced to a single population of cells until they become metabolically inactive or until the highest concentration has been introduced.

Test Article and Positive Control Preparation

- On the day of dosing, the test article is suspended in the experimental medium.
- Serial dilutions of the test article (7-9 doses) are prepared for exposure to the cells.
- Sodium Lauryl Sulfate (SLS) (stock concentration 100 mg/ml in sterile water) is used as the positive control and it is diluted into seven concentrations for dosing.

Data Evaluation

- Calculate the MRD_{50} (the concentration which caused 50% inhibition of the basal acidification rate).