

# MTT Time Course Assay with EpiDerm™ Cultures

**Theory:** The purpose of this assay is to evaluate the potential dermal irritancy of a test article to the EpiDerm™ construct (MatTek Corporation) as determined by the exposure time (or concentration) of a test article required to reduce cell viability to 50% of control viability. Cell viability is measured by the NAD(P)H-dependent microsomal enzyme reduction of MTT (and to a lesser extent, the succinate dehydrogenase reduction of MTT) (3[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) to a blue formazan precipitate, in treated cultures, and is expressed as a percentage relative to untreated (negative control) cultures<sup>1</sup>.

## Applications and Use

- The EpiDerm™ model is composed of human keratinocytes stratified into a 3-dimensional dermal structure consisting of basal, spinous, and granular layers, including a functioning stratum corneum with characteristic lipid lamellae.
- EpiDerm™ is suited to address the sensitivity range from very mild to severely aggressive, or corrosive materials.
- Test materials are applied topically at formulation strength.
- Suited for both water soluble and insoluble formulations.
- Suitable for testing creams, pastes, highly viscous materials, and powders otherwise precluded from testing in other models.
- Cells are of human origin and can be induced to express and generate inflammatory response cytokines.

## Experimental Procedure

### **Receipt and Preparation of Cultures**

- Each culture is removed with sterile forceps from the agarose gel, inspected, and transferred to a pre-labeled 6-well plate containing 0.9 mL of assay medium per well. The EpiDerm™ cultures will be incubated at 37±1°C in a humidified atmosphere of 5±1% CO<sub>2</sub> in air for at least one hour prior to dosing.

### **Assay Procedure**

- The test materials are tested NEAT by topical application. Pastes and highly viscous materials may be "creamed" to effect application.
- The positive control is 1.0% Triton®-X-100 (a surfactant) exposed for 4 and 8 hours.
- Positive controls for modeling inflammatory responses (Croton oil, PMA, DNCB, etc.) may be included.
- The negative control is sterile, deionized water generally exposed concurrently with the longest and shortest exposure times of the test or positive control articles.
- 100 µL (liquids) or 30 mg (solids) of the test or control article are applied topically onto the tissue surface.
- The cultures are returned to the incubator for the appropriate exposure times. Generally, a minimum of four exposure times, ranging from 30 minutes to 24 hours, are selected. For addressing severely irritating or corrosive materials, exposure times of 1 to 60 minutes may be selected.
- After the appropriate exposure time, the test articles are rinsed from the cultures using DPBS without Ca<sup>++</sup> and Mg<sup>++</sup>.
- The cultures are transferred to wells containing 0.3 mL of MTT reagent (1 mg/mL) and incubated for 3 hours.
- After incubation, the cultures are blotted on absorbent paper and extracted in 2 mL of isopropanol for 2 hours, with shaking.
- 200 µL of each extraction solution are transferred to a 96-well plate and the absorbance at 550nm (OD<sub>550</sub>) recorded.
- Medium samples may be collected and prepared for a variety of cytokine analyses to assess inflammatory responses.

## Data Evaluation

- The relative survival is determined by comparing the mean corrected OD<sub>550</sub> of the test article-treated wells to the mean corrected OD<sub>550</sub> of the negative control-treated wells.
- Exposure time response curves may be plotted with the % of control on the ordinate and the test article exposure times on the abscissa.
- The ET<sub>50</sub> (the time of exposure to the test article which reduces MTT conversion by 50%) is determined by interpolation from the exposure time response curves.
- Occasionally, a test article may directly reduce the MTT giving erroneous results. A direct MTT reduction test is performed as a pre-screen, and "killed tissue" controls may be assayed concurrently.
- **Interpretation:** Exposure response curves and ET<sub>50</sub> values are compared between test formulations as well as benchmarks, if applicable. MatTek Corporation provides a Draize prediction model for interpreting a range of chemical classes.

<sup>1</sup> Berridge, M.V., Tan, A.S., McCoy, K.D., Wang, R. (1996) The Biochemical and Cellular Basis of Cell Proliferation Assays That Use Tetrazolium Salts. **Biochemica** 4:14-19.