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**Tradename:** AC Party Face Pom

**Code:** 20447

**CAS #:** 7732-18-5 & 84961-57-9 & 8028-48-6

**Test Request Form #:** 14248

**Lot #:** N250903D

**Sponsor:** *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
**Study Director:** *Daniel Shill*
**Principal Investigator:** *Hannah Stade*

**Test Performed:**
In Vitro EpiDerm™ Model (EPI-200-SIT) Phototoxicity

## SUMMARY

*In vitro* phototoxicity irritation studies were conducted to evaluate whether **AC Party Face Pom** would induce phototoxic irritation in the EpiDerm™ model assay.

The assay was performed according to the manufacturer's protocol. The test article solution was found to be a non-photoirritant at 0.5%, 1.5%, 5.0% and 10.0% concentrations. Reconstructed human epidermis were incubated in growth media for one hour to allow for tissue equilibration. The test substance was applied to the tissue inserts in four varying concentrations and incubated overnight in a humidified incubator. The following day, the appropriate tissue inserts were irradiated (UVA) for 60 minutes with 1.7 mW/cm$^2$ (=6 J/cm$^2$). After substance incubation, irradiation, and washing was completed, cell viability was assessed. Cell viability was measured by dehydrogenase conversion of MTT [(*3-4,5-dimethyl thiazole 2-yl*)], present in the cell mitochondria, into blue formazan salt that was measured after extraction from the tissue. The photoirritation potential of the test chemical was dictated by the reduction in tissue viability of UVA exposed tissues compared to non-UVA exposed tissues.

Under the conditions of this assay, the test article was considered to be non-phototoxic at concentrations of 0.5%, 1.5%, 5.0%, and 10.0%. The negative and positive controls performed as anticipated.

## Introduction

A. Purpose

An *in vitro* dermal phototoxicity study was conducted to evaluate whether a test article would induce photoirritation in the EpiDerm™ model assay. MatTek Corporation's reconstructed human epidermal model is becoming a standard in determining the phototoxicity potential of a test substance. This assay is able to discriminate between photoirritants and non-photoirritants at varying concentrations.

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## Materials

| | |
|---|---|
| A. Incubation Conditions: | 37°C, 5% $CO_2$, and 95% relative humidity (RH) |
| B. Equipment: | Forma humidified incubator, ESCO biosafety laminar flow hood, Synergy HT Microplate reader; UVA-vis Irradiation Equipment; UVA meter; Pipettes |
| C. Media/Buffers: | Dulbecco's Modified Eagle Medium (DMEM) based medium; sterile Dulbecco's phosphate-buffered saline (DPBS); sterile deionized water $H_2O$ ($diH_2O$) |
| D. Preparation: | Pre-incubate (37°C) tissue inserts in assay medium; Place assay medium and MTT diluent at 4°C, MTT concentrate at -20°C, and record lot numbers of kit components |
| E. Tissue Culture Plates: | Falcon flat bottom 96-well, 24-well, and 6-well tissue culture plates |
| F. Reagents: | MTT (*3-4,5-dimethyl thiazole 2-yl*) (1.0 mg/mL); Extraction Solution (Isopropanol); Chlorpromazine; Triton X-100 (1.0%) |
| G. Other: | Wash bottle; sterile disposable pipette tips; Parafilm; forceps |

## Test Assay

A. Test System

The reconstructed human epidermal model, EpiDerm™ consists of normal human-derived epidermal keratinocytes which have been cultured to form a multilayer, highly differentiated model of the human epidermis. This model consists of organized basal, spinous, and granular layers, and contains a multilayer stratum corneum containing intercellular lamellar lipid layers. The EpiDerm™ tissues are cultured on specially prepared cell culture inserts. The EpiDerm™ tissue inserts from MatTek were in good condition, intact, and viable upon arrival.

B. Positive and Negative Controls

a. Positive Control: Concentrations of chlorpromazine, ranging from 0.001% to 0.1%, were used as positive controls for the EpiDerm™ Phototoxicity assay.

b. Negative Control: $diH_2O$ was used as the negative control for the EpiDerm™ Photoxicity assay.

C. Data Interpretation Procedure

A photoirritant is predicted if the mean relative tissue viability of the 2 tissues exposed to the test substance and 60 minutes of 6 J/$cm^2$ is reduced by 20% compared to the non-irradiated control tissues.

## Method

A. Tissue Conditioning

Upon arrival, the EpiDerm™ tissue inserts were removed from their shipping medium and transferred into new culture plates with fresh media and incubated at 37°C, 5% $CO_2$, and 95% RH for 60 minutes. After the 60 minute incubation, the inserts were transferred into new culture plates with fresh media and dosing commenced.

B. Test Substance Exposure and Tissue Washing

a. Exposure: 50 µL of the diluted test substance in their respective concentrations were applied to 2 tissue inserts and allowed to incubate for overnight at 37°C, 5% $CO_2$, and 95% RH.

b. Washing: After UVA-irradiation and dark incubation, the tissue inserts were washed using DPBS and transferred to new culture plates with fresh media for an overnight incubation at 37°C, 5% $CO_2$, 95% RH.

C. MTT Assay

Tissue inserts were transferred into 300 µL MTT media in pre-filled plates and incubated for 3 hours at 37°C, 5% $CO_2$, and 95% RH. Next, inserts were placed into sealed culture plates with 2 mL of the extraction solution and incubated in the dark for 24 hours at room temperature. Following this incubation, extraction was complete and the tissue inserts were pierced with forceps. Duplicate 200 µL aliquots of the blue formazan solution from each tissue insert was transferred into a 96-well plate for Optical Density reading at a wavelength of 570 nm ($OD_{570}$) with a spectrophotometer.

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Acceptance Criterion

A. Positive and Negative Control
   a. Positive Control: The assay meets acceptable criterion if a dose dependent reduction in cell viability in the UVA-irradiated tissues is between 0.00316% and 0.0316%.
   b. Negative Control: The results are acceptable if the mean negative control Optical Density ($OD_{570}$) is ≥ 0.8.

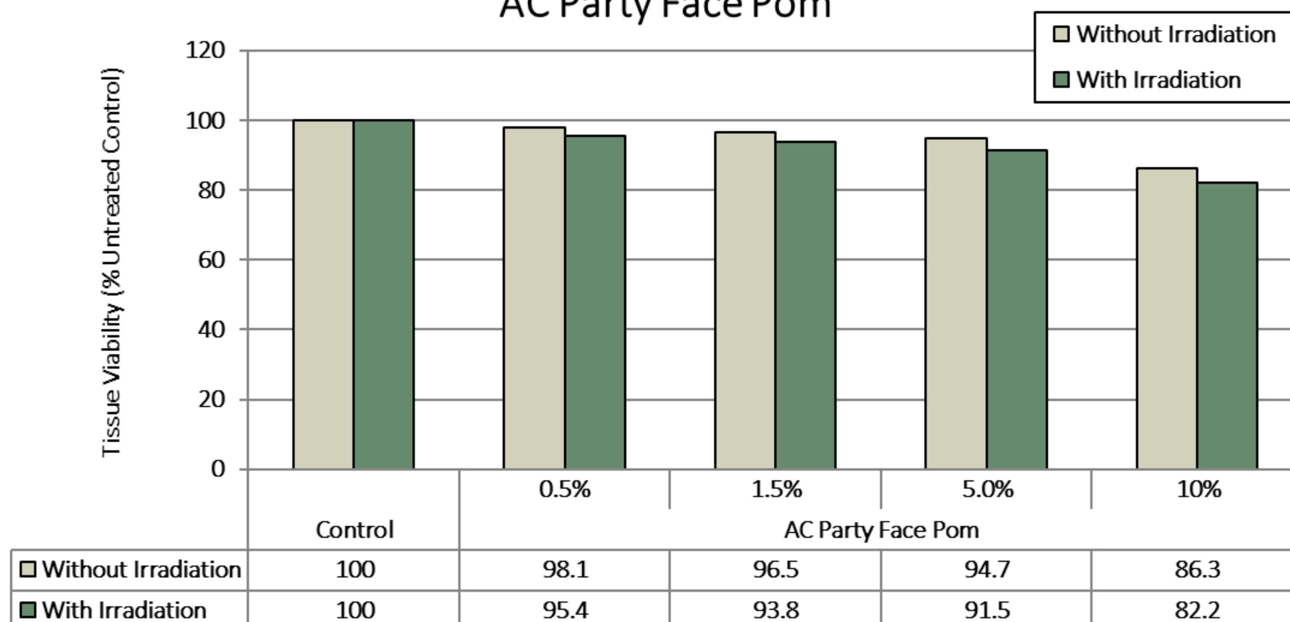
B. Standard Deviation
   Provided phototoxicity potential is predicted from the mean viability of 2 tissues for the EpiDerm™ Phototoxicity Protocol, the variability of the replicates should not exceed 30%.

Results

A. Test Validity and Tissue Viability Assay
   The data obtained from this study met criteria for a valid assay. The results are summarized in Figure 1. Cell viability was calculated for each tissue as a percentage of the corresponding vehicle control either irradiated or non-irradiated. Tissue viability was not reduced by 20% in the presence of the test substance and UVA-irradiation at concentrations of 0.5%, 1.5%, and 5.0%. The negative control mean exhibited acceptable relative tissue viability while the positive control exhibited dose dependent loss of tissue viability and cell death.

Conclusion

Phototoxicity (photoirritation) is defined as an acute toxic response that is elicited after exposure of the skin to certain chemicals and subsequent exposure to light. Under the conditions of this assay, the test article substance was considered to be non-phototoxic at concentrations of 0.5%, 1.5%, 5.0%, and 10.0%. The negative and positive controls performed as anticipated. There is a slight decrease in viability at the 10% concentration but viability does not decrease more than the acceptable 20%. We can safely say that **AC Party Face Pom** is not a photoirritant when used at the suggested use levels of 2.0% - 5.0%.




| | Control | 0.5% | 1.5% | 5.0% | 10% |
|---|---|---|---|---|---|
| Without Irradiation | 100 | 98.1 | 96.5 | 94.7 | 86.3 |
| With Irradiation | 100 | 95.4 | 93.8 | 91.5 | 82.2 |

**Figure 1.** EpiDerm™ Phototoxicity Graph.

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