# Evaluation of TRPV1 Activity to Assess the Eye Stinging Potential of Cosmetic Formulations

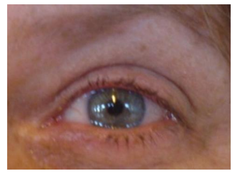
Arvind Gill[1], Wei Chen[1], Kimberly Norman[2], Lindsay Krawiec[2], Dang Alphonsus[1] and Cristi Gomez[1]

[1] Department of Product Safety, Mary Kay Inc., Dallas, TX, USA
[2] Institute for In Vitro Sciences, Inc., Gaithersburg, MD, USA

## ABSTRACT

The Transient Receptor Potential Vanilloid type 1 (TRPV1) receptor is one of the most well characterized pain-inducing receptors and has been recently identified as a valuable tool to predict eye stinging potential of surfactant based formulations. In this study we sought to predict eye stinging of non-surfactant based cosmetic formulations by studying TRPV1 activity using the NociOcular assay. In the NociOcular assay, TRPV1 expressing neuroblastoma cells are exposed to test substance and TRPV1 activity is measured by acute increases in intracellular calcium. Three of the formulations induced stinging in the human test and were also positive in the NociOcular assay. The other four formulations evaluated were classified as stinging in the human test, but a conclusive determination could not be made in the NociOcular assay as the formulations were not fully soluble in assay buffers. The formulations were also evaluated in the EpiOcular™ assay, an established *in vitro* model for eye irritation utilized by the cosmetics industry. The Epiocular™ assay results did not correlate with the human sting data. Our data support that the NociOcular assay may be a valuable *in vitro* tool to predict human eye stinging sensation for cosmetic formulations. Future efforts seeks to further expand the applicability of the assay to product types other than surfactant based formulations.

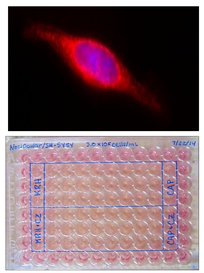
## EXPERIMENTAL PLAN



- The NociOcular assay has previously been shown to predict the eye stinging potential of surfactant based formulas. Our goal was to determine if the NociOcular assay could assess eye stinging potential for non-surfactant based formulas.
- Seven prototype personal care formulas with varying degrees of eye stinging potential were selected for evaluation based on $ET_{50}$ scores from the EpiOcular™ eye irritation assay and/or clinical ocular testing.
- Since these formulas are non-surfactant based, we sought to establish appropriate solvent(s) for use in the assay and dilution scheme(s) which were more relevant to these formulas.
- EpiOcular™ assay ($ET_{50}$ scores) and/or clinical results and classification were compared with NociOcular assay results.

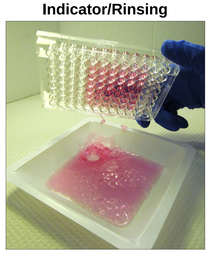
## NOCIOCULAR *IN VITRO* ASSAY

**Seeding**



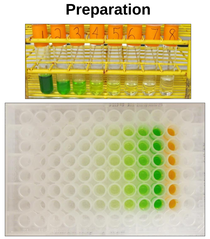
**Step 1**: TRPV1 transfected SH-SY5Y cells are seeded in 96-well plates and incubated until an appropriate confluency is achieved.

**Addition of Calcium Indicator/Rinsing**



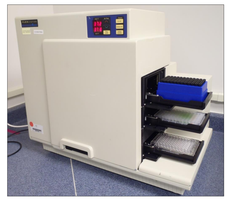
**Step 2:** The cells are treated with a calcium dye indicator and rinsed twice prior to the addition of assay buffer. Half of the wells receive buffer with a TRPV1 antagonist (capsazepine)

**Test Article Preparation**



**Step 3:** A dilution of the test article is prepared and added to a 96-well compound plate that is later used for dosing. The compound plate also contains the solvent control (assay buffer) and the positive control (TRPV1 agonist) for comparison.

**Dose and Read Plate**



**Step 4:** A cell plate, compound plate, and tips are loaded into the FlexStation Fluorometer. The cell plate is systematically dosed and the fluorescence intensity is recorded using SoftMax Pro software. The data is saved and analyzed using SoftMax Pro, Microsoft Excel, and Prism software.

## BACKGROUND

Evaluating ocular irritation is essential to the safety assessment of facial and eye-area cosmetic products. Both *in vitro* and clinical testing methods are extensively used by the personal care industry to evaluate products for eye irritation and eye sensory response. The EpiOcular™ assay is one of the most commonly used *in vitro* assays by the personal care industry because it is a sensitive tissue model which identifies eye irritation potential. The key parameter involved in EpiOcular™ screening assay is the $ET_{50}$, which is the time required for the product tested to reduce the tissue viability (as measured by the MTT) to 50%, as compared to control treated tissues. The $ET_{50}$ was chosen because it is an indirect measure of the tissue barrier properties. Eye irritation potential is assessed according to $ET_{50}$ value; however, no correlation has been established between the $ET_{50}$ value and sensory response such as eye stinging.

Human clinical studies are a valuable approach to capture both ocular irritation and ocular comfort, including redness, stinging, itching, burning, and eye tearing. However, human clinical testing is only applicable for confirmed mild ingredients or products and limited data is available. In addition to eye irritation, sensory eye response is an important factor for consumer use of personal care products. Therefore, an *in vitro* model capable of identifying the ocular sensory response as a prescreening tool, especially during the formula development phase, would be greatly beneficial.

The Transient Receptor Potential Vanilloid type 1 (TRPV1) receptor is one of the most well characterized pain-inducing receptors. A novel *in vitro* method, NociOcular assay, was recently developed and has been used to predict eye stinging potential for surfactant based formulations. Considering many personal care products are non-surfactant based, an attempt was made to evaluate if NociOcular assay could also be expanded to non-surfactant based formulas for predicting eye stinging potential.

**Table 1. Eye Irritation Assessment for each Product using the EpiOcular™ Screening Assay.** Each product was evaluated in the EpiOcular™ screening assay and an $ET_{50}$ value determined. The $ET_{50}$ value was then used to determine an irritancy rating based on Mary Kay internal assessment scales for each product type. The clinical testing results for eye stinging do not correlate with the $ET_{50}$ value and irritancy classification.

| Product Code | EpiOcular™ $ET_{50}$ | Eye Irritation Classification | Clinical Testing Results |
|---|---|---|---|
| Facial Mask (Clay) | < 0.5 hour | Irritating | Mild Eye Sting |
| Facial Mask 01 | < 1 hour | Irritating | Eye Sting |
| Eye Make-up Remover | 1.5 hours | Irritating | NA |
| Facial Mask 02 | 7.3 hours | Non-irritating, minimal | Mild Eye Sting |
| Eye Cream 01 | > 24 hours | Non-irritating, minimal | Erythema; Eye Sting |
| Eye Cream 02 | > 24 hours | Non-irritating, minimal | Eye Sting |
| Eye Gel | > 24 hours | Non-irritating, minimal | Eye Sting |

## NOCIOCULAR DATA ANALYSIS

- Each formulation was assessed for $Ca^{2+}$ influx over a range of formulation concentrations. The $Ca^{2+}$ influx was compared to the capsaicin response (set to 100%).
- For each formulation an Emax value (% of capsaicin response) was determined.
- To ascertain that the $Ca^{2+}$ influx was due to TRPV1 specific activation, the receptor antagonist, capsazepine, was added to the cells prior to formulation dilutions.
- The seven formulations were ranked according to TRPV1 specific activity using the Emax value and capsazepine responses.

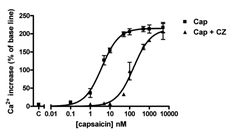
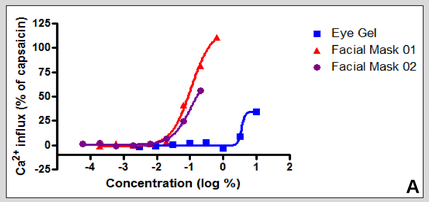



**Figure 1.** Capsaicin-induced concentration-effect curve of $Ca^{2+}$ in TRPV1-SH-SY5Y cells as measured with the $Ca^{2+}$-binding and fluorescent probe Fura-2/AM. (■) 10 μM capsazepine was added to the wells before capsaicin addition and measurements (▲).

## RESULTS




| Product Code | Human Clinical Testing Results | TRPV1 specific response | Emax |
|---|---|---|---|
| Facial Mask 01 | Eye Stinger | Yes | 118.2 |
| Facial Mask 02 | Mild Eye Stinger | Yes | 76.8 |
| Eye Gel | Eye Stinger | Yes | 34.5 |
| Eye Make-up Remover | NA | No | NA |

B

**Figure 2. Eye-Stinging Comparison of Formulations Soluble in KRH buffer**. KRH buffer is the standard test substance diluent in the NociOcular assay. Only 4/7 formulations were soluble in KRH buffer. The graph shows the concentration effect curves of the 3 formulations which showed TRPV1 specific responses. **(A)**. The range of responses are graded by color ranging from red (strongest TRPV1 response) to blue. The table **(B)** displays the clinical testing result, determination of TRPV1-specific effect, and Emax values observed for each formulation. Products are ranked according to specific TRPV1 activity, as measured by the Emax value and comparison to treatment with receptor antagonist, capsazepine (not shown).

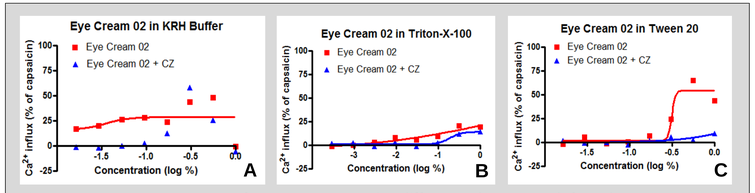



**Figure 3. Dose-Response Curves for Eye Cream 02 using different solvents.** Each formulation was assessed for solubility prior to the assay. Since KRH buffer is the traditional test substance diluent in the assay, it was first attempted to use KRH buffer as the diluent even if solubility was limited. Eye Cream 02 was first evaluated in the assay using KRH buffer (A) and no dose response was observed. Next, Triton X-100, 0.1% in KRH (B) and Tween 20, 0.2% in KRH (C) were used as solvents. Dilution in 0.2% Tween 20 increased solubility a TRPV1 specific response was observed.

**Table 2. Assessment of Insoluble Formulations.** Three formulations were not aqueous soluble and therefore had limited solubility using KRH buffer. Each formulation, except Facial Mask (Clay), was evaluated using KRH buffer, 0.1% Triton X-100, and 0.2% Tween 20 as the formulation diluent. In 0.2% Tween 20 formulation solubility was improved and a TRPV1 Specific response was observed for Eye Cream 01 an 02.

| Product Type | Clinical Testing Results | NociOcular assay Solvents | Specific TRPV1 response | Emax |
|---|---|---|---|---|
| Eye Cream 02 | Eye Sting | KRH<br>0.1% Triton X-100<br>0.2% Tween 20 | No<br>No<br>Yes | NA<br>NA<br>54.4 |
| Eye Cream 01 | Erythema; Eye Sting | KRH<br>0.1% Triton X-100<br>0.2% Tween 20 | No<br>No<br>Yes | NA<br>NA<br>44.5 |
| Facial Mask (Clay) | Mild Eye Sting | KRH<br>0.1% Triton X-100<br>0.2% Tween 20 | No<br>Not determined; insoluble<br>Not determined; insoluble | NA<br>NA<br>NA |

## CONCLUSIONS & NEXT STEPS

- Of the seven formulations, 4/7 were soluble in KRH buffer (typical buffer used for surfactant based products) and 3/4 were identified as having TRPV1 specific activity which correlated with the clinical results for those 3 formulations.
- Technical challenges with solubility were encountered for 3/7 formulations and alternate solvents including 0.1% Triton X-100 and 0.2% Tween 20 were used to improve solubility.
- Using Tween 20 as solvent improved solubility and a TRPV1 specific response was observed for 2/3 insoluble formulations.
- No solvent was found to be compatible with the Facial Mask (Clay), and further investigation will be conducted
- Overall, the results of the NociOcular Assay appeared to correlate with the clinical ocular testing for sensorial response and we seek to expand the dataset and further evaluate use of the NociOcular assay as a screening tool for personal care product eye stinging.