**Background Information**

- Cyprotex's ocular irritation test is performed using MatTek's in vitro 3D EpiOcular™ model. Cyprotex are a MatTek-approved laboratory for performing these studies.

- The EpiOcular™ tissue model consists of normal, human-derived epidermal keratinocytes that have been cultured to form a stratified, squamous epithelium which closely mimics the human corneal epithelium.

- EpiOcular™ was developed by MatTek to create an in vitro (non-animal) alternative to the animal-based Draize Eye Irritation Test used in the cosmetics, personal care, household products, chemical, pharmaceutical, and biotech industries.

- The EpiOcular™ eye irritation test model is currently undergoing ECVAM validation. Peer-reviewed publications have shown the EpiOcular™ eye irritation model achieves greater than 80% accuracy when compared with the Draize eye irritation test.

- The EpiOcular™ model is metabolically and mitotically active and produces the same cytokines involved in irritation and inflammation in vivo.

- A range of different test articles can be assessed including liquids, solids, semi-solids, pastes, gels, creams and waxes.

- Cyprotex's ocular irritation test is based upon assessment of the cytotoxicity following exposure to a test chemical, typically at three time points. Cytotoxicity is expressed as a decrease in mitochondrial conversion of MTT to formazan.

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**Related Services**

Skin corrosion  
Skin irritation  
Phototoxicity  
Skin absorption

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**Protocol**

**Method Available**
EpiOcular™ Eye Irritation Test (ET<sub>50</sub> determination)

**Model Used**
MatTek EpiOcular™ 3D human tissue model  
(others available on request)

**Number of Concentrations**
Typically 1 (depending on client needs)

**Number of Replicates**
1 - 3 (depending on assay and client needs)

**Exposure Times**
3 - 5 (depending on assay and client needs)

**Controls**
Positive Control = between 1-10% Benzalkonium chloride  
Negative control = Sterile ultrapure water  
Reference control = 10% Baby shampoo (optional)

**Endpoints**
MTT  
IL-1α release (optional)  
Histology (optional)

**Additional endpoints available:**
- cytokine release (IL-6, IL-8, TNFα)  
- oxidative stress  
- glutathione levels  
- mitochondrial function

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'The EpiOcular-EIT, together with EpiOcular's long history of reproducibility and proven utility for ultra-mildness testing, make EpiOcular a useful model for addressing current legislation related to animal use in the testing of potential ocular irritants.'

Kaluzhey Y et al., (2011) Altern Lab Anim 39(4); 339-364
‘Damage to the corneal epithelium resulting from exposure to chemicals and mixtures of chemicals may compromise tissue function, and can result in various effects ranging from mild irritation, to the loss of cornea transparency or blindness.’

Figure 1
Graph illustrating the cell viability over time using the EpiOcular™ EIT model. Error bars represent ± SEM (n=3).

Table 1
The ET<sub>50</sub> and estimated Draize modified maximum average scores (MMAS) were determined using the cell viability data. Based on these results, the Predicted Potency was determined and compared to actual potency from various published reports.

Table 2
Proposed method for classification of ocular irritation potency.

Figure 2
Structural similarity between EpiOcular™ 3D tissue model and the rabbit cornea.

References
1 Pfannebecker U et al., (2013) Cosmetics Europe multi-laboratory pre-validation of the EpiOcular™ reconstituted human tissue test method for the prediction of eye irritation. Toxicology In Vitro 27(2): 619-626