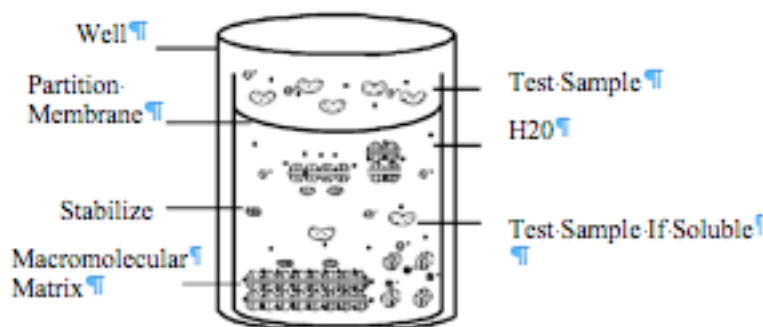


## InVitro Ocular and Dermal Irritation Assays

The proprietary Ocular and Dermal Irritation assays are standardized and quantitative *in vitro* acute ocular and dermal irritation tests, which utilize changes of relevant macromolecules to predict acute ocular and dermal irritancy of chemicals and chemical formulations.

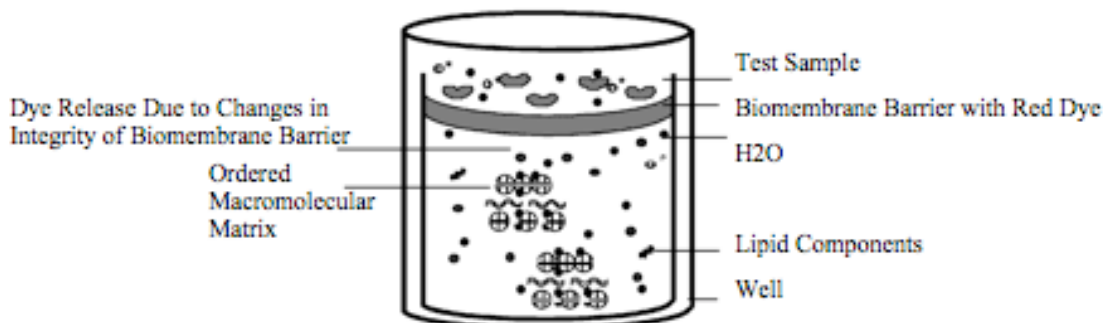
The Ocular Irritation assay, depicted schematically in Figure 1 below, provides significant advances over the *in vivo* Draize test method. The Draize eye irritation assay has been criticized because of the large variability of results obtained from different laboratories that have analyzed the same specimen.

**Figure 1.** The Ocular Irritation Model



The Dermal Irritation assay, depicted schematically in Figure 2, is based on the principle that chemical compounds will promote measurable changes in target biomolecules and macromolecular structures. Previous studies have clearly demonstrated that the processes of protein denaturation and disaggregation that are induced in this *in vitro* assay mimic the effects that are produced when these types of irritants are applied to the skin. Consequently, this *in vitro* test may be employed to predict the *in vivo* toxic effects of chemicals and formulations.

**Figure 2.** The Dermal Irritation Model



The quantitative Ocular and Dermal Irritation *in vitro* assays have been found to be highly reproducible. Of even greater relevance, the Ocular and Dermal Irritation assay methods can be readily employed to evaluate multiple samples at varying volumes or concentrations. Thus, these tests serve as extremely useful screening tools that facilitate all stages of raw material selection, formulation development and final product selection.

### **Materials/Methods**

The Ocular and Dermal Irritation assays are quantitative *in vitro* test methods that mimic acute ocular and dermal irritation tests. To perform the Ocular Irritation standardized assay, the test sample is applied to a synthetic biobarrier composed of a semi-permeable membrane. To perform the Dermal Irritation standardized assay, the test sample is applied to a similar synthetic biobarrier that is coated with a dye-containing keratin-collagen matrix. Following application, the sample is absorbed by and permeates through this synthetic biobarrier to gradually come into contact with a proprietary solution containing highly ordered globulins and glycoproteins. Reaction of the test sample with these proteins and macromolecular complexes promotes conformational changes that may be readily detected as an increase in the turbidity of the protein solution. With the Ocular Irritation test, turbidity may be detected spectrophotometrically at a wavelength of 405 nm. With the Dermal Irritation test, dye that has been dissociated from the biobarrier during transit of the applied sample may be detected spectrophotometrically at a wavelength of 450 nm.

The ocular irritancy potential of a test sample is expressed as an Irritation Draize Equivalent (IDE), whereas the dermal irritancy potential of a test sample is expressed as a Human Irritancy Equivalent (HIE) score. These scores are defined by comparing the increase in optical density (OD<sub>405/450</sub>) produced by the test material to a standard curve that is constructed by measuring the increase in OD produced by a set of Calibration substances. These Calibrators have been selected for use in these tests because their irritancy potential has been previously documented in a series of *in vivo* investigations. The predicted *in vivo* classification, based on these scoring systems, is shown in Tables 1 and 2.



3904 Del Amo Blvd Torrance, CA 90503  
Phone: (310) 214-0043 Fax: (310) 370-3642  
www.bioscreen.com Email: info@bioscreen.com

**Table 1.** Relationship of Irritation Draize Equivalent (IDE) Score to Irritancy Classification for the Ocular Irritation Test Method.

Irritation Draize Equivalent (IDE) Score	Predicted Ocular Irritancy Classification
0.0 - 12.5	Minimal Irritant
12.5 - 30.0	Mild Irritant
30.0 - 51.0	Moderate Irritant
51.0 - 80.0	Severe Irritant

**Table 2.** Relationship of Human Irritancy Equivalent (HIE) Score to Irritancy Classification for the Dermal Irritation Test Method.

Human Irritancy Equivalent (HIE)	Predicted Dermal Irritancy Classification
0.00 - 0.90	Non-Irritant
0.90 - 1.20	Non-Irritant/Irritant
1.20 - 5.00	Irritant

Last update: July 2010