



# Dermal Irritation Services

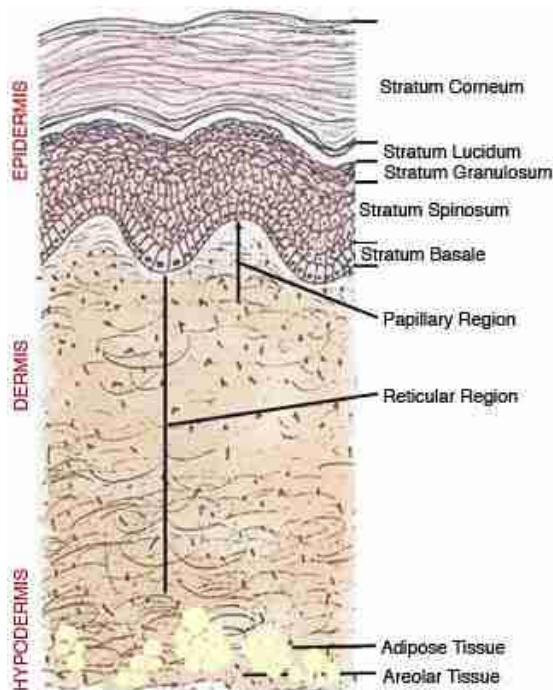


Leading the Field of *In Vitro* Toxicology

CeeTox is pleased to offer a series of 3-dimensional models that test for dermal irritation on both individual ingredients and finished products. They accurately assess dermal irritation for the majority of liquids, solids, semi-solids, pastes, gels, creams and waxes.

CeeTox routinely uses the SkinEthic RHE™ (SkinEthic Corporation) model and the EpiDerm™ EPI-200 (MatTek Corporation) model for dermal irritation testing. However, we have additional cell models available upon request by our clients

The *in vitro* skin irritation tests offered by CeeTox allow you to rapidly meet your regulatory and safety testing needs, and comply with the European Union Cosmetic Directive, Amendment 7, and REACH requirements (see table on right).



Source: <http://www.lionden.com/SkinLayers.jpg>  
Used with permission.

CeeTox is a GLP compliant laboratory, therefore assays may be performed and reported in a GLP compliant manner if desired by the client.

**Dermal Irritation** refers to the production of reversible damage to the skin following the application of a test substance. The principle of the *in vitro* dermal irritation models is that test articles that penetrate the stratum corneum and are cytotoxic to the cells in the underlying layers are classified as irritants.

The cytotoxic effect of test articles on viable cells in the basal layer underlying the stratum corneum is determined by a colorimetric MTT assay. In this assay, MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, thiazolyl blue) is reduced to formazan salt by cellular mitochondrial dehydrogenases active only in living cells. The reduction of cell viability in treated tissues is compared to negative controls and expressed as a percentage.

If the cytotoxic effect as measured by the MTT assay is absent or weak, assays measuring Interleukin 1 alpha (IL-1 $\alpha$ ) may be run. IL-1 $\alpha$  is a cytokine that is released into the media during an inflammatory response. Human keratinocytes express and release large amounts of IL-1 $\alpha$ . Other cytokines or inflammatory markers can also be tested, including but not limited to interleukins, chemotactic cytokines, growth-promoting factor, transforming growth factor, cytokines regulating humoral versus cellular immunity and other signaling factors.

In addition, histology can be performed to visualize the morphological changes to the cells caused by the test compounds. The histological evaluation improves interpretations and reduces false negatives.

Reporting may take three basic forms. Our standard report includes data charts and graphs detailing results of the assays run. A detailed report is optionally available as well. This report includes:

- Executive Summary
- Objective
- Experimental Design
- Results
- Tables and Figures
- Materials and Methods
- Appendix (if necessary)

Finally, a report complying with GLP requirements is available for those studies performed according to GLP regulations.

CeeTox has a number of irritation assays for you to choose from.

MODEL	EpiSkin™ R38 Reference	EpiDerm™ Standard Skin Irritation Test	EpiDerm™ Skin Irritation Range Finding Assay	SkinEthic RHE™ Skin Irritation Test	EpiDerm™ Combined Skin Irritation and Corrosion Test
<b>Purpose of Assay</b>	Distinguishes between R38 skin irritating and (no label) non-skin irritating substances.	Distinguishes between R38 skin irritating and (no label) non-skin irritating substances.	This assay uses the standard MatTek protocol but uses 3-5 exposure times to add additional understanding of the potential for skin irritation over a longer exposure time than 1 hour.	Distinguishes between R38 skin irritating and (no label) non-skin irritating substances.	This assay assesses both irritation and corrosion of a test article.
<b>Cell Model</b>	EpiSkin (SkinEthic Laboratories)	EPI-200 (MatTek Corporation)	EPI-200 (MatTek Corporation)	RHE (SkinEthic Laboratories)	EPI-200 (MatTek Corporation)
<b>Assays Performed</b>	<ul style="list-style-type: none"> <li>• MTT reduction</li> <li>• IL-1<math>\alpha</math> release (optional)</li> <li>• H&amp;E stained histology slide (optional)</li> </ul>	<ul style="list-style-type: none"> <li>• MTT reduction</li> <li>• IL-1<math>\alpha</math> release (optional)</li> <li>• H&amp;E stained histology slide (optional)</li> </ul>	<ul style="list-style-type: none"> <li>• MTT reduction</li> <li>• IL-1<math>\alpha</math> release (optional)</li> <li>• H&amp;E stained histology slide (optional)</li> </ul>	<ul style="list-style-type: none"> <li>• MTT reduction</li> <li>• IL-1<math>\alpha</math> release (optional)</li> <li>• H&amp;E stained histology slide (optional)</li> </ul>	<ul style="list-style-type: none"> <li>• MTT reduction</li> <li>• IL-1<math>\alpha</math> release (optional)</li> <li>• H&amp;E stained histology slide (optional)</li> </ul>
<b>Controls</b>	MTT direct reduction, freeze-killed (if needed), no application, positive, negative; R38 reference	MTT direct reduction, freeze-killed (if needed), no application, positive, negative	MTT direct reduction, freeze-killed (if needed), no application, positive, negative	MTT direct reduction, freeze-killed (if needed), no application, positive, negative	MTT direct reduction, freeze-killed (if needed), no application, 1 positive for irritation, 1 positive for corrosion, negative
<b>Exposure</b>	15 minutes	60 minutes	3-5 exposures from 1 hour to 48 hours	42 minutes	3 minutes / 60 minutes
<b>Incubation</b>	42 hours	42 hours	42 hours	42 hours	42 hours
<b>Number of Replicates</b>	3	3	3	3	3
<b>Standard Turnaround Time</b>	2 weeks from sample and tissue receipt	2 weeks from sample and tissue receipt	2 weeks from sample and tissue receipt	2 weeks from sample and tissue receipt	2 weeks from sample and tissue receipt
<b>Scientific Endorsement</b>	ECVAM	ECVAM	ECVAM (for standard protocol)	ECVAM	ECVAM
<b>International Regulatory Acceptance</b>	Draft OECD Test Guideline	Draft OECD Test Guideline	Draft OECD Test Guideline plus adds extra time points	Draft OECD Test Guideline	Draft OECD Test Guideline OECD for irritation and Test Guideline 431 for corrosion
<b>National Regional Regulatory Acceptance</b>	EU test method B.46 in COM regulation 440/2008/EC	EU test method B.46 in COM regulation 440/2008/EC	Uses same protocol as EU test method B.46 in COM regulation 440/2008/EC	EU test method B.46 in COM regulation 440/2008/EC	EU test method B.46 in COM regulation 440/2008/EC

References:

Harvell, J.D., Lammintausta, K, Maibach H.I. (1995) Irritant contact dermatitis in: Guin J.D. Practical Contact Dermatitis McGraw-Hill New York, 7-18.

Hayden, Patrick (2007, December 6) The Way Forward for In Vitro Skin Irritation Testing from <http://www.alttox.org/trc/toxicity-tests/skin-irritation/way-forward/hayden/>

OECD Guideline for the testing of chemicals, Draft proposal for a new guideline, In vitro skin irritation: Human skin model test from <http://www.oecd.org/dataoecd/21/56/40793105.doc>

