1. The submitted test method and supporting validation data should have been subjected to a transparent and independent peer review process.

   This is an in vitro skin irritation test method using the 3D human skin model EpiSkin that can be used to predict skin irritation potency in rabbits or as an alternative to the Draize skin irritation test. This test method has been reviewed by the ECVAM Scientific Advisory Committee (ESAC), which has published a *Statement on the Scientific Validity of in-Vitro Tests for Skin Irritation Testing*. In this statement, ESAC concluded that this test conformed to OECD GD34 and was a suitable alternative.

   Based on the above, we conclude that the test method has been subjected to a transparent and independent review process.

   * Test method B.4, per OECD TG 404 and Annex V of the EU Dangerous Substance Directive 67/548/EEC.

2. The data generated by the test method should adequately measure or predict the endpoint of interest. For replacement test methods, the data should show a linkage between the proposed test method and an existing test method, and/or the proposed test method and effects in the target or model species.

   Skin irritation potency is a characteristic of substances that induce reversible inflammation through direct application to the surface of the skin.

   Reactions that result in skin irritation occur when an irritating substance is absorbed by and diffused through the stratum corneum, thereby affecting the underlying cells.

   EpiSkin is a skin model that comprises skin cells beneath a stratum corneum, for which viability is indexed after exposure to a test substance.

   Inflammatory reaction is confirmed by measuring inflammatory cytokines (IL-1α).

   Therefore, the data obtained from this test method is similar to that of earlier test methods in that it is a measurement of reactions like those made with Draize skin irritation test using animals.

3. The test method should generate data useful for hazard/risk assessment purposes.

This test method is useful as a skin irritation test used to distinguish the skin irritation potency of chemical substances. It is capable of predicting the results of skin irritation tests used to distinguish the skin irritation potency of chemical substances. It was used to evaluate the molecular structure, substituent groups, and physiochemical properties of 58 substances known to exhibit hazardous properties, and results were in agreement for 49 of 58 substances (84.5%), based on median values.

Therefore, based on the submitted data, this test method is useful for assessing hazards from exposure to chemical substances.

* Test method B.4, per OECD TG 404 and Annex V of the EU Dangerous Substance Directive 67/548/EEC.

4. The submitted test method and supporting validation data should adequately cover a spectrum of chemicals and products representative of those administered by the regulatory program or agency for which the test method is proposed, and the applicability and limitations of the test method should be clearly described.

The chemical substances tested using this test method comprised 58 substances selected by the Chemicals Selection Sub-committee (CSSC), classified as follows.

EU label classification:	25 R38 labels, 33 no-labels
GHS classification:	13 irritants, 17 mild irritants, 28 non-irritants
Combined EU-GHS:	13 R38-I, 12 R38-MI, 5 no–label-MI, and 28 no–label-NI

Restrictions to application come from the use of MTT reduction, which means that the results from this test method could be impaired by the use of colored substances or reducing agents.

These substances exhibit a variety of molecular structures, substituent groups, and physiochemical properties, but the purpose of their use in a product was not specified. Therefore the scope of application for a particular regulatory program or agency was not clear.

5. The test method should be sufficiently robust (relatively insensitive to minor changes in protocol) and transferable among properly-equipped laboratories with adequately-trained staff.

The results from three different laboratories were in agreement for 50 of 58 substances (86.2%), indicating no issues with inter-laboratory reproducibility and that this technology is highly transferable.
This test method has a well-defined protocol and is robust as long as test parameters are satisfied.

This test method can be performed using ordinary culturing equipment and techniques. Training is required for techniques involving the collection of punch biopsies after addition of MTT dyes and cell proliferation.

6. The test method should be both time and cost effective as well as likely to be used in a regulatory context.

The three-day's time, including preculture, required to perform this test method is relatively short, making it time effective.
The cost of human skin models is approximately 100,000 JPY per 12 wells.

7. Justification should be provided (scientific, ethical, economical) for the new or updated test method in light of existing test methods.

Scientific justification is available from the fact that replication of inflammatory reactions are confirmed.

Validity as an alternative method that contributes to animal welfare comes from the fact that no animals are used.

One difficulty with using this method in Japan comes from the fact that it requires time to obtain but cannot be stored for long periods of time after purchase.

This is a market product and therefore attention must be paid to changes in manufacturing processes and availability of a stable supply, but its use as can be justified as an alternative test method.

8. The test method should be suitable for use as regulatory documentation in the assessment of safety.

This test method assesses skin irritation potential after a four-hour application of a chemical substance. Within that limitation, it is suitable for used in a regulatory

context.

This test method has not been evaluated for use in assessing skin irritation after a twenty-four–hour application of a chemical substance, as is required for quasi-drugs and cosmetic products.

Based on the above, the JaCVAM Regulatory Acceptance Board has determined the following.

The alternative test method for skin irritation potency using human skin models (EpiSkin 3D skin model) is an ethically-justified test method, which if used under suitable conditions, is capable of providing scientific assessment of the general skin irritation potency of chemical substances.