

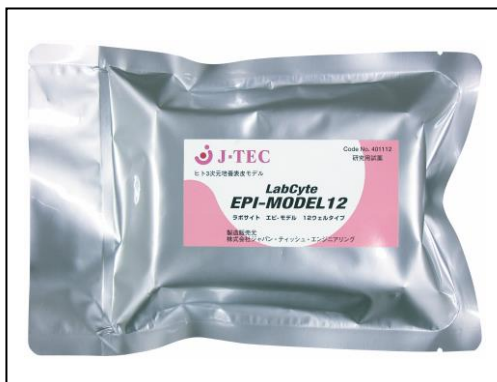


## LabCyte EPI-MODEL

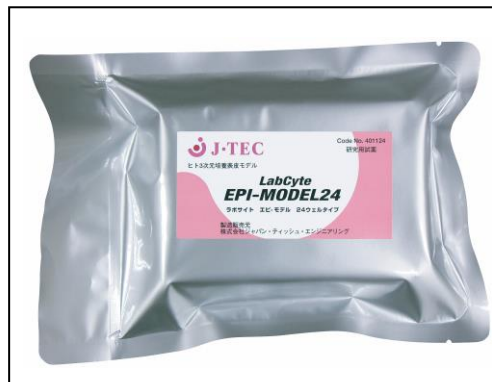
Three-dimensional Cultured Human Epidermis Model

### User's Manual

For Research Use Only



**EPI-MODEL 12**



**EPI-MODEL 24**

**Please read this manual before use**

**Updated March, 2017**

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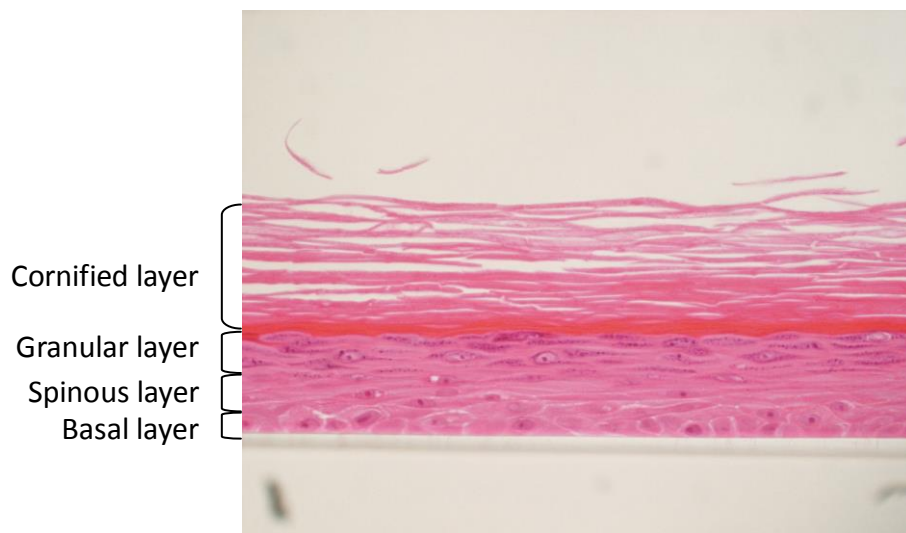
Thank you for purchasing LabCyte EPI-MODEL (3-D Cultured Human Epidermis Model). Please read this manual before use.

## I. Characteristics of LabCyte EPI-MODEL

LabCyte EPI-MODEL is a 3-dimensional reconstructed human epidermis model, produced by culturing normal human epidermal cells to become multilayered. The structure of LabCyte EPI-MODEL is morphologically similar to that of human epidermis, consisting of the basal layer, the spinous layer, the granular layer and the cornified layer.

LabCyte EPI-MODEL can be used as an alternative to experimental animals in skin irritation tests, and human epidermis-related studies such as toxicology, pharmacology, and dermatology.

LabCyte EPI-MODEL is produced with J-TEC's advanced epidermal culture techniques, which enables the gain of highly reproducible results in evaluation tests, with little lot-to-lot variation.



Hematoxylin-eosin staining of LabCyte EPI-MODEL tissue section

## II. Kit components

The LabCyte EPI-MODEL kit includes the following components:

### 1. 3-D Cultured Human Epidermis Model: 1 plate



Each LabCyte EPI-MODEL plate is individually packed in aluminum bags.

The culture inserts containing human epidermal tissues are fixed to each well of the plate by nutritive agar.

The number of culture inserts included in one plate is as follows:

- EPI-MODEL 12: 12 inserts
- EPI-MODEL 24: 24 inserts

### 2. Assay Medium: 30ml



### 3. Assay Plate: 1 plate



12-Well plate



24-Well plate

The culture inserts containing human epidermal tissues fixed in nutritive agar medium are transferred to this plate.

- EPI-MODEL 12: 12-well plate
- EPI-MODEL 24: 24-well plate

### **III. Handling Suggestions**

#### **1. Product handling upon delivery**

When you receive the LabCyte EPI-MODEL package, make sure there is no leakage or damage on the product.

Please contact us at the following address if you find any defects.

Japan Tissue Engineering Co., Ltd.

Sales and Marketing Department

TEL: +81-533-66-2129    FAX: +81-533-66-2018

e-mail:jtec-info@jpte.co.jp

#### **2. Storage and shelf life**

Store unopened LabCyte EPI-MODEL aluminum packages at room temperature. The expiration date is written on the label of the aluminum package. For better reproducibility of results, it is recommended to keep the storage period prior product use the same across experiments.

It is recommended that all culture inserts are used once the package is opened. Do not store this product in the provided assay medium, or in a CO<sub>2</sub> incubator (37°C, 5-10% CO<sub>2</sub>), as cellular metabolism is activated under these conditions.

The assay medium should be stored in a refrigerator (2 to 8°C). The expiration date of the medium is written on the label of the bottle. The assay medium contains serum, which may result in floating residues and aggregates. These do not interfere with the quality and performance of the product.

## IV. How to Use

An example of the tests and studies that can be performed using the LabCyte EPI-MODEL is described in this section. LabCyte EPI-MODEL has a tissue structure that is similar to that of the epidermis, and can be used for different purposes.

### ***in vitro* Skin Irritation Test protocol**

#### **1. Equipment required**

- Safety or laminar flow cabinet
- Water bath
- CO<sub>2</sub> incubator (37°C, 5-10% CO<sub>2</sub>)
- 96-well multi-plate reader (540 to 590 nm, 650 nm)

#### **2. Other supplies required**

- Assay medium (supplied or sold separately)
- Assay plate (supplied or sold separately)
- MTT (sold separately)
- 96-well plate (sold separately)
- Isopropanol
- Phosphate buffered saline (PBS)
- Sterile forceps
- Sterile pipette
- Microtubes

#### **3. Assay plate preparation**

*All manipulations should be aseptically performed in a safety or laminar flow cabinet.*

1. Warm up the assay medium to 37°C using a water bath.
2. Fill the required number of wells of the assay plate with the following amount of warm assay medium:

**EPI-MODEL 12: 1 ml / well**

**EPI-MODEL 24: 0.5 ml / well**

3. Open the aluminum package containing the human epidermis model.
4. Open the plate lid and pick up the culture inserts using sterile forceps.

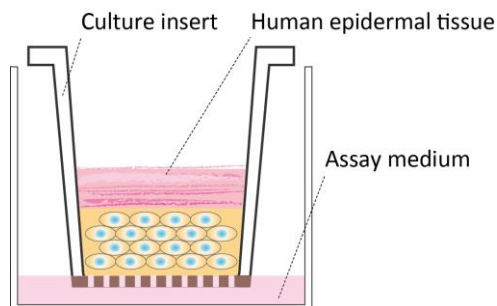


The culture inserts can be easily picked if air is allowed in between the bottom of the culture insert and the agar medium.

**Note:**

*Use a pair of sterile forceps to remove agar medium sticking to the outside wall of the culture inserts.*

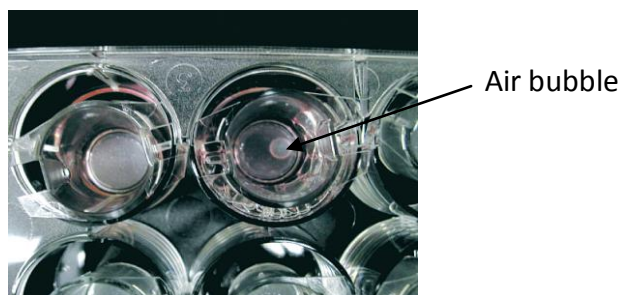
5. Transfer the culture inserts into wells of the assay plate prepared on step 2.



**Note:**

*Avoid air bubble under the culture inserts. Air bubbles can be removed by shaking the culture insert using forceps.*





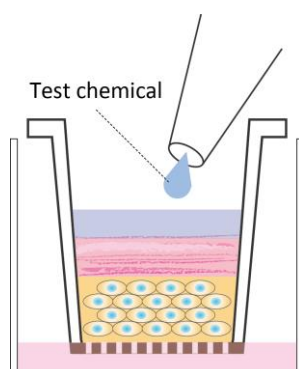
6. Close the lid of the assay plate and place it in a CO<sub>2</sub> incubator.
7. Incubate for at least 1 hour before the application of any test chemicals.

#### 4. Application of Test Chemicals

*All manipulations should be aseptically performed in a laminar flow or safety cabinet.*

1. Take the assay plate from the CO<sub>2</sub> incubator.
2. Apply the required amount of test chemicals onto the surface of the epidermal tissues, and spread it over the entire epidermal surface. Depending on the experimental design, some culture inserts should be left empty, or filled with the test chemical solvent or carrier as a negative control.

**The recommended sample volume of test chemical is 100 µl for EPI-MODEL 12, and 50 µl for EPI-MODEL 24. The maximum volume capacity is 1.5 ml for EPI-MODEL 12, and 0.8 ml for EPI-MODEL 24. Highly viscous or solid chemicals should be weighed with a balance prior application. Apply these chemicals with a spatula, taking care not to scratch the surface of the model.**



3. Close the lid of the assay plate and place it in a CO<sub>2</sub> incubator.
4. Incubate for the required time period according to the experimental design.

## 5. MTT Assay

*All manipulations should be aseptically performed in a laminar flow or safety cabinet.*

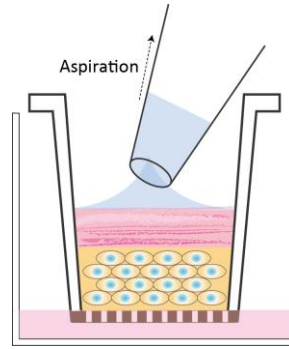
1. Prepare MTT medium by dissolving MTT (3-(4,5-dimethylthiazol-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) in the assay medium (final concentration: 0.5mg/ml). Sterilize the MTT medium by filtering it through a 0.22 µm or a 0.45 µm filter if necessary (sterilization is not required when the reaction time is shorter than 24 hours). Prepare the MTT medium immediately prior to use. Store the MTT medium in a cool and dark place and use it within 24 hours. Pre-warm the medium in a 37°C water bath for 1 hour prior to use.
2. Prepare isopropanol for dye extraction.
3. Take the assay plate treated with the test chemicals from the CO<sub>2</sub> incubator.
4. Remove the test chemicals from the culture inserts by aspiration.

**For EPI-MODEL 24, the test chemical can be easily removed by using a tapered Pasteur pipette, or an aspirating pipette attached with a micropipette tip that can fit in the small diameter of the culture insert.**

### **Note:**

*Be careful not to damage the surface of the epidermis.*

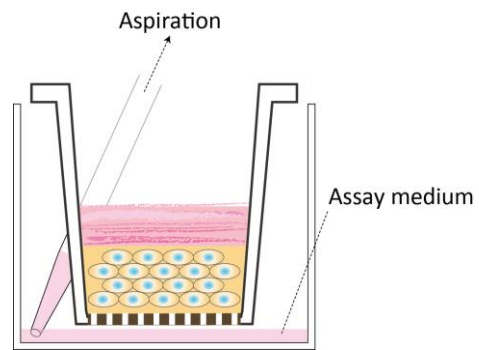
5. Wash the EPI-MODEL by filling up the culture inserts with phosphate buffered saline (PBS) and discarding buffer. To remove any residual test chemical, repeat this washing step for at least three times. The washing method varies according to the nature of the test chemical being used.



**Note:**

*Do not directly squirt the epidermal surface with phosphate buffered saline (PBS), or the epidermal tissue could detach from the bottom of the culture insert.*

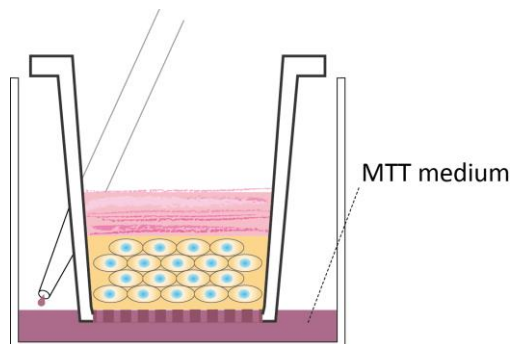
6. Remove by aspiration any medium from the outside wall of the culture inserts.



7. Fill each well with the following amount of warm MTT medium:

**EPI-MODEL 12: 1 ml/well**

**EPI-MODEL 24: 0.5 ml/well**

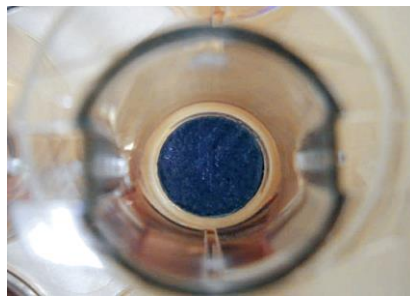


8. Close the lid of the assay plate and place it in a CO<sub>2</sub> incubator.
9. Incubate for 3 hours.

#### 6. MTT Extraction and Measurement of Optical Density

*The following procedures do not require working under aseptic conditions.*

1. Take the assay plate from the CO<sub>2</sub> incubator and make sure that tissues not treated with any test chemicals (negative control) are stained purple.



2. Remove the human epidermal tissues from the culture inserts using forceps.

**If the epidermal tissues cannot be removed by forceps due to damage caused by the test chemicals, use a spatula to scrape the tissue off of the membrane, or use a scalpel to cut the membrane filter from the base of the culture insert.**



3. Transfer each epidermal tissue to a microtube.
4. Add isopropanol into the micro-tube and immerse the entire epidermal tissue in it.



**The amount of extraction solvent is as follows:**

**EPI-MODEL 12: 300 to 500  $\mu$ l**

**EPI-MODEL 24: 200 to 300  $\mu$ l**

5. Incubate the microtubes in a dark place at room temperature for more than 2 hours (no longer than 24 hours) in order to extract the dye. If a longer solubilization step is required, tightly seal the microtubes to avoid dye volatilization.
6. Transfer the extracted solution from the microtubes into separate wells of a 96-well plate. Prepare one well with isopropanol as blank.

**The recommended volume for optical density measurement is 200  $\mu$ l for EPI-MODEL 12, and 150  $\mu$ l for EPI-MODEL 24.**

7. Measure optical density (OD) at 540 to 590 nm (570 nm recommended) using a 96-well plate reader. More precise data can be obtained by subtracting OD at 650 nm from OD at 540 to 590 nm (570 nm).
8. Calculate cell viability (%): OD of epidermal tissue treated with the test chemical against that of the negative control.

$$\text{Cell Viability (\%)} = \frac{\text{OD}_{\text{test chemical}} - \text{OD}_{\text{blank}}}{\text{OD}_{\text{negative control}} - \text{OD}_{\text{blank}}} \times 100$$

## V. Basic Precautions

- Use this product for research purposes only.
  
- Under any circumstance, do not apply this product to humans or animals, and do not use it for *in vitro* diagnostic procedures.
  
- Although virus screening is conducted (HIV, HBV, HCV, and HPV), this product should be used with extreme caution.
  
- It is recommended to conduct preliminary tests with the intended test chemical prior use.
  
- J-TEC assumes no responsibility for any accidents or damage resulting from the use of this product for purposes other than those originally intended.
  
- No return or exchange will be accepted after receipt of the product in any of the following cases.
  - 1) Damage caused by improper handling;
  - 2) Damage caused by carelessness handling; and
  - 3) Damage caused by natural disasters such as fire, earthquake, flood and lightning, and other unavoidable circumstances.
  
- Discard used products after adequate processing.





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