J-TEC

LabCyte CORNEA-MODEL

Three-dimensional Cultured Human Cornea Epithelial Model

User's Manual

For Research Use Only

Please read this manual before use

Updated March, 2017

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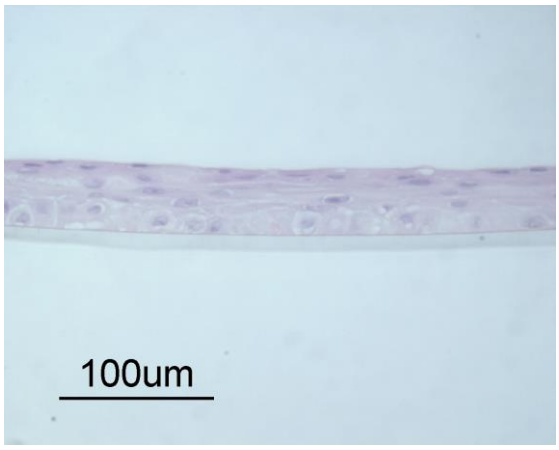
Thank you for purchasing LabCyte CORNEA-MODEL (3D Cultured Human Cornea Epithelial Model). Please read this manual before use.

I. Characteristics of LabCyte CORNEA-MODEL

LabCyte CORNEA-MODEL is a 3D human cultured cornea epithelial tissue produced from normal human cornea epithelial cells. LabCyte CORNEA-MODEL was developed by applying cell culture techniques to differentiate and stratify cornea epithelial cells to form a tissue structure similar to that of the normal human cornea. LabCyte CORNEA-MODEL can be used in the hazard identification of irritant chemicals by the eye irritation test. Moreover, CORNEA-MODEL can also be used to evaluate biological processes in response to different test substances.



Hematoxylin-Eosin staining of LabCyte CORNEA-MODEL

II. Kit components

The LabCyte CORNEA-MODEL kit included the following components:

1. 3D Cultured Human Cornea Epithelial Model: 1 plate

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

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

24 culture inserts are included in one plate.

2. Assay Medium: 30 ml

3. Assay plate: 1 plate

III. Handling Suggestions

1. Product handling upon delivery

When you receive the LabCyte CORNEA-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 CORNEA-MODEL aluminum packages at room temperature. The expiration date is described 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_2 incubator (37˚C, 5-10% CO_2), 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 CORNEA-MODEL is described in this section. LabCyte CORNEA-MODEL has a tissue structure that is similar to that of the human cornea epithelium, and can be used for different purposes.

IV-1. *in vitro* Eye Irritation Test protocol

1. Equipment required

- Safety or laminar flow cabinet
- Water bath
- CO_2 incubator (37˚C, 5-10% CO_2)

2. Other supplies required

- Assay medium (supplied or sold separately)
- 24-well assay plate (supplied or sold separately)
- 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 0.5 ml of warm assay medium.

3. Open the aluminum package containing the human cornea epithelial model.

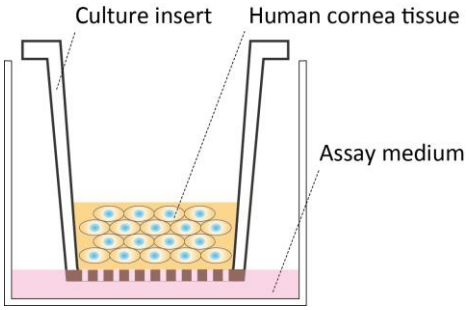
4. Open the plate lid and pick up the culture inserts using sterile forceps.

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

Note:

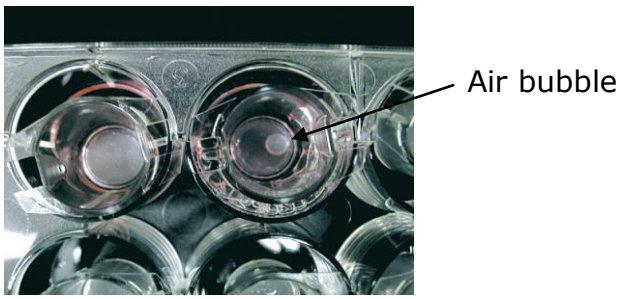
Use a pair of sterile forceps to remove agar medium sticking to the outside wall of the culture inserts.

5. Transfer the culture inserts into the 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 plate it in a CO_2 incubator.

7. Incubate for at least 1 hour before the application of any test chemicals.

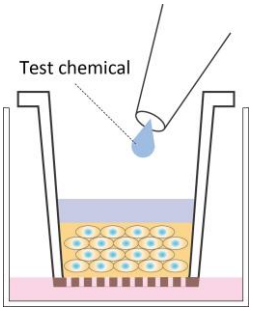
4. Application of test chemicals

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

1. Take the assay plate from the CO_2 incubator.

2. Apply the required amount of test chemicals onto the surface of the tissues, and spread it over the entire 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 50 µl. The maximum volume capacity is 0.8 ml. 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_2 incubator.

4. Incubate for the required time period according to the experimental design.

5. Rinsing of test chemicals

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

1. Take the assay plate treated with the test chemicals from the CO_2 incubator.

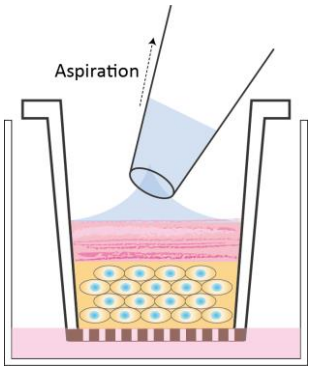
2. Remove the test chemicals from the culture inserts by aspiration.

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 cornea epithelium.

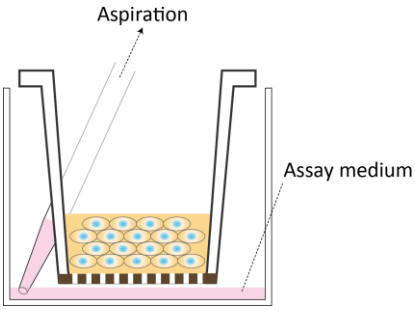
3. Wash the CORNEA-MODEL by filling up the culture inserts with phosphate buffered saline (PBS) and discarding the buffer. To remove any residual test chemical, repeat this washing step for at least three times. The washing method varies according to the nature of the test chemical being used.



Note:

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

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



5. Proceed to section IV-2. CORNEA-MODEL culture protocol, if necessary.

6. Alternatively, after step 4, proceed to section IV-3. MTT Assay, or section IV-4 WST8 Assay to determine the number of viable cells and the irritancy of the test chemical. Optionally, process the tissues for histological observation, or cytokine measurements.

IV-2. CORNEA-MODEL culture protocol

1. Equipment required

- Safety or laminar flow cabinet
- Water bath
- CO_2 incubator (37˚C, 5-10% CO_2)

2. Other supplies required

- Assay medium (supplied or sold separately)
- 24-well assay plate (supplied or sold separately)
- Phosphate buffered saline (PBS)
- Sterile forceps
- Sterile pipette

3. Procedure

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

1. Warm up the assay medium to 37˚C using a water bath.

2. Remove the medium from the wells by aspiration.

3. Add 0.5 ml of warm medium to each well.

4. Close the lid of the assay plate and place it in a CO_2 incubator.

5. Change the medium everyday, and culture the models for the required time period according to the experimental design.

IV-3. MTT Assay

1. Equipment required

- Safety or laminar flow cabinet
- Water bath
- CO_2 incubator (37˚C, 5-10% CO_2)
- 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. Procedure

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: 0.5 mg/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. Remove by aspiration any medium from the outside wall of the culture inserts.

3. Fill each well with the 0.5 ml of warm MTT medium.

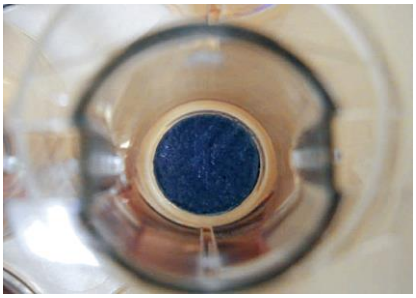
4. Close the lid of the assay plate and place it in a CO_2 incubator.

5. Incubate for 3 hours.

4. 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_2 incubator and make sure that tissues not treated with any test chemicals (negative control) are stained purple.

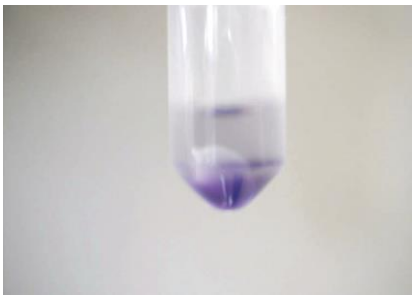


2. Remove the tissues from the culture inserts using forceps.

If the 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 corneal tissue to a microtube.

4. Add isopropanol into the micro-tube and immerse the entire corneal tissue in it.



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 150 µl.

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 cornea epithelial tissue treated with the test chemical against that of the negative control.

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

IV-4. WST-8 Assay

1. Equipment required

- Safety or laminar flow cabinet
- Water bath
- CO_2 incubator (37˚C, 5-10% CO_2)
- 96-well multi-plate reader (450 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)
- Phosphate buffered saline (PBS)
- Sterile forceps
- Sterile pipette

3. Procedure

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

1. Prepare WST-8 solution by dissolving WST-8 in PBS or culture medium at 1:10. Warm up the WST-8 solution in a water bath 37˚C prior use.

2. Add 0.3 µl of WST-8 solution to wells in a fresh 24-well plate.

3. Hold the culture insert with a pair of forceps and wash its outside walls in a culture dish (or any other shallow container) containing PBS. Repeat this procedure and dry the culture insert with a paper towel.

4. Transfer the culture insert to the plate containing WST-8 prepared in step 2. Prepare the blank by leaving a well without a culture insert.

5. Close the lid of the assay plate and place it in a CO_2 incubator.

6. Incubate for 3-4 hours.

4. MTT Extraction and Measurement of Optical Density

The following procedures do not require working under aseptic conditions.

1. Take the assay plate from the shaker incubator. Make sure that the solution of the negative control is orange.

2. Mix the WST-8 solution by pipetting and transfer 200 µl to a well in a 96-well plate.

3. Measure optical density (OD) at 450 nm using a 96-well plate reader. More precise data can be obtained by subtracting OD at 650 nm from OD at 450 nm.

4. Calculate cell viability (%): OD of cornea epithelial tissue treated with the test chemical against that of the negative control.

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

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, and HCV), 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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