eurofins | BioPharma Product Testing

# *In vitro* Eye Irritation:

# Ocular Irritation Assay using the EpiOcular™ Human Tissue Model

# with

# PC-BC01

**Report**

**Version: Final**

**Eurofins Munich Study No.: 187785**

**Sponsor:**
ProCell Therapeutics
#1009, Ace-Twin Tower II,
273, Digital-ro, Guro-gu,
Seoul,
Korea

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GLP / GMP FDA approved

# 1. Copy of the GLP Certificate

Bayerisches Landesamt für
Gesundheit und Lebensmittelsicherheit

## GLP-Bescheinigung/Statement of GLP Compliance
(gemäß/according to § 19b Abs. 1 Chemikaliengesetz)

Eine GLP-Inspektion zur Überwachung der Einhaltung der GLP-Grundsätze gemäß Chemikaliengesetz bzw. Richtlinie 2004/9/EG wurde durchgeführt in:

Assessment of conformity with GLP according to Chemikaliengesetz and Directive 2004/9/EC at:

☒ Prüfeinrichtung/Test facility ☐ Prüfstandort/Test site

**Eurofins BioPharma Product**
**Testing Munich GmbH**
Behringstraße 6-8
82152 Planegg
(Unverwechselbare Bezeichnung und Adresse/Unequivocal name and address)

Prüfungen nach Kategorien/Areas of Expertise
(gemäß/according ChemVwV-GLP Nr. 5.3/OECD guidance)

| | |
|---|---|
| **Kategorie 2** | **Category 2** |
| **Kategorie 3** | **Category 3** |
| **Kategorie 8** | **Category 8** |
| **Kategorie 9*** | **Category 9*** |
| *Sonstige Prüfungen:* Biologische und mikrobiologische Sicherheitsprüfungen an Medizinprodukten und Arzneimitteln; Auftragsarchivierung | *other tests:* biological and microbiological safety evaluation on medical devices and pharmaceuticals; contract archiving |

Datum der Inspektion/Date of Inspection
(Tag.Monat.Jahr/day.month.year)

**15.03.2018**

Die/Der genannte Prüfeinrichtung/Prüfstandort befindet sich im nationalen GLP-Überwachungsverfahren und wird regelmäßig auf Einhaltung der GLP-Grundsätze überwacht.

The above mentioned test facility/test site is included in the national GLP Compliance Programme and is inspected on a regular basis.

Auf der Grundlage des Inspektionsberichtes wird hiermit bestätigt, dass in dieser Prüfeinrichtung/diesem Prüfstandort die oben genannten Prüfungen unter Einhaltung der GLP-Grundsätze durchgeführt werden können.

Based on the inspection report it can be confirmed, that this test facility/test site is able to conduct the aforementioned studies in compliance with the Principles of GLP.

Schwabach, 26.04.2018

***Dr. Peter Franke***
**Leiter der GLP-Landesleitstelle Bayern**

GLP- Landesleitstelle Bayern
Bayerisches Landesamt für Gesundheit
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# 2. Contents

# 3. List of Tables

# 4. Preface

## 4.1. Abbreviations

| | |
|---|---|
| Aqua dest. | Aqua destillata *(distilled water)* |
| Art. | Artikel *(article)* |
| BGBl. | Bundesgesetzblatt *(Federal Law Gazette)* |
| COLIPA | European Cosmetics Association |
| DMEM | Dulbecco's Modified Eagle Medium |
| DPBS | Dulbecco's phosphate buffered saline |
| e.g. | exempli gratia *(for example)* |
| ECVAM | European Centre for the Validation of Alternative Methods |
| EIT | Eye Irritation Test |
| EURL ECVAM | European Union Reference Laboratory for Alternatives to Animal Testing |
| Eurofins Munich | Eurofins BioPharma Product Testing Munich GmbH |
| GLP | Good Laboratory Practice |
| GmbH | Gesellschaft mit beschränkter Haftung *(company with limited liability)* |
| I | irritant |
| KCCV | killed control corrected viability |
| KT | test item treated killed tissues |
| KU | negative control of killed tissues |
| MTT | 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide |
| NI | non-irritant |
| NC | negative control of living tissues |
| No. | number |
| $NSC_{living}$ | non-specific colour of additional viable tissues |
| $NSC_{killed}$ | non-specific colour of additional killed tissues |
| NSCCV | NSC-corrected mean relative tissue viability |
| NSMTT | non-specific reduction of MTT |
| OD | optical density |
| $OD_{net}$ | net optical density |
| OECD | Organisation for Economic Co-operation and Development |
| PBS | phosphate buffered saline |
| QA | Quality Assurance |
| QAU | Quality Assurance Unit |
| RhCE | Reconstructed human cornea-like epithelium |
| SOP | Standard Operating Procedure |
| TKT | additional test item treated killed tissue without MTT staining |
| TM | test item treated living tissues |
| TVT | additional test item treated living tissue without MTT staining |
| UN GHS | United Nations Globally Harmonized System on the Classification and Labelling of Chemicals |

## 4.2. General

| | |
|---|---|
| Sponsor: | ProCell Therapeutics<br>#1009, Ace-Twin Tower II,<br>273, Digital-ro, Guro-gu,<br>Seoul,<br>Korea |
| Study Monitor: | MyeongSeop Song<br>Biotoxtech Co., Ltd.<br>53 Yeongudanji-ro,<br>Ochang-eup, Cheongwon-gu,<br>Cheongji-si,<br>Chungcheongbuk-do<br>Korea |
| Test Facility: | Eurofins BioPharma<br>Product Testing Munich GmbH<br>Behringstraße 6/8<br>82152 Planegg<br>Germany |
| Eurofins Munich Study No.: | 187785 |
| Test Item: | PC-BC01 |
| Title: | *In vitro* Eye Irritation: Ocular Irritation Assay using the EpiOcular™ Human Tissue Model with PC-BC01 |

## 4.3. Project Staff

| | |
|---|---|
| Study Director: | Dr. Helge Gehrke |
| Team Leader<br>Operational QA GLP: | Uwe Hamann |

## 4.4. Schedule

| | |
|---|---|
| Arrival of the Test Item: | 24 October 2018 |
| Study Initiation Date: | 12 November 2018 |
| Experimental Starting Date: | 14 November 2018 |
| Experimental Completion Date: | 22 November 2018 |
| Study Completion Date: | Date of the study director's signature |

# 5. Quality Assurance

## 5.1. GLP Compliance

This study was conducted to comply with:

Chemikaliengesetz ("Chemicals Act") of the Federal Republic of Germany, Appendix 1 to § 19a as amended and promulgated on July 18, 2017 (BGBl. I S. 2774) [1].

Konsens-Dokument der Bund-Länder-Arbeitsgruppe Gute Laborpraxis ("Consensus Document of the National and Länder Working Party on Good Laboratory Practice") on the archiving and storage of records and materials, 5 May 1998 [2].

OECD Principles of Good Laboratory Practice (as revised in 1997); OECD Environmental Health and Safety Publications; Series on Principles of Good Laboratory Practice and Compliance Monitoring - Number 1. Environment Directorate, Organisation for Economic Co-operation and Development, Paris 1998 [3].

The OECD Principles of Good Laboratory Practice are accepted by regulatory authorities throughout the European Community, USA and Japan.

This study was assessed for compliance with the study plan and the Standard Operating Procedures of Eurofins Munich. The study and/or the test facility are inspected periodically by the Quality Assurance Unit according to the corresponding SOPs. These inspections and audits are carried out by the Quality Assurance Unit, personnel independent of staff involved in the study. A signed quality assurance statement, listing all performed audits, is included in the report.

## 5.2. Guidelines

This study followed the procedures indicated by internal Eurofins Munich SOPs and the following internationally accepted guidelines and recommendations:

OECD Guideline for the Testing of Chemicals No. 492: Reconstructed human Cornea-like Epithelium (RhCE) test method for identifying chemicals not requiring classification and labelling for eye irritation or serious eye damage, 25 Jun 2018 [4].

EURL ECVAM DB-ALM Method Summary No. 164: EpiOcular™ Eye Irritation Test - Summary, 22 July 2015 [5]

EpiOcular™ Eye Irritation Test (OCL-200-EIT) For the prediction of acute ocular irritation of chemicals For use with MatTek Corporation's Reconstructed Human EpiOcular™ Model, 29 June 2015 [6].

### 5.3. Archiving

For a period of 15 years (or shorter if in compliance with the GLP regulations) Eurofins Munich will store the records, materials and specimens in their scientific archives according to the GLP regulations.

The following records have to be stored according to the GLP regulations:

A copy of the final report, the study plan and documentation of all raw data generated during the conduct of the study (documentation forms as well as any other notes of raw data, printouts of instruments and computers) and the correspondence with the sponsor concerning the study. Any document relating to the study will be discarded only with the prior consent of the sponsor.

The following materials and samples have to be stored according to the period of time specified in the GLP regulations:

A retained sample of the test item will be archived according to the GLP regulations, if possible, and will be discarded without the sponsor's prior consent.

Other materials and specimens have to be stored according to the GLP regulations and disposed of after the respective archiving period with the sponsor's prior consent.

Unless otherwise agreed in writing, the remaining test item will be discarded three months after the release of the report.

# 6. Statement of Compliance

| | |
|---|---|
| Eurofins Munich Study No.: | 187785 |
| Test Item: | PC-BC01 |
| Title: | *In vitro* Eye Irritation: Ocular Irritation Assay using the EpiOcular™ Human Tissue Model with PC-BC01 |
| Study Director: | Dr. Helge Gehrke |

This study performed in the test facility Eurofins Munich was conducted in compliance with Good Laboratory Practice Regulations:

Chemikaliengesetz ("Chemicals Act") of the Federal Republic of Germany, Appendix 1 to § 19a as amended and promulgated on July 18, 2017 (BGBl. I S. 2774) [1].

Konsens-Dokument der Bund-Länder-Arbeitsgruppe Gute Laborpraxis ("Consensus Document of the National and Länder Working Party on Good Laboratory Practice") on the archiving and storage of records and materials, 5 May 1998 [2].

"OECD Principles of Good Laboratory Practice (as revised in 1997)", Paris 1998 [3].

There were no circumstances that may have affected the quality or integrity of the study.

Study Director: Dr. Helge Gehrke

.....

Date: 22 Nov 2019

# 7. Statement of the Quality Assurance Unit

| | |
|---|---|
| Eurofins Munich Study No.: | 187785 |
| Test Item: | PC-BC01 |
| Title: | *In vitro* Eye Irritation: Ocular Irritation Assay using the EpiOcular™ Human Tissue Model with PC-BC01 |
| Study Director: | Dr. Helge Gehrke |

This report and the conduct of this study were inspected by the Quality Assurance Unit on the following dates:

| Phase of QAU Inspection | Date of QAU Inspection | Date of Reporting to the Study Director and Management |
|---|---|---|
| Audit Final Study Plan: | 12 November 2018 | 12 November 2018 |
| Audit Experimental Phase (process-based): | 25 October 2018 | 25 October 2018 |
| Audit Final Report: | 22 MAR 2019 | 22 MAR 2019 |

This report reflects the raw data.

Member of the
Quality Assurance Unit:

M. Rasch

Print Name: Michaela Rasch

Date: 22 MAR 2019

# 8. Summary

## 8.1. Summary Results

In the present study the eye irritating potential of PC-BC01 was analysed. Since irritant substances are cytotoxic to the corneal epithelium after a short time exposure the cytotoxic effects of the test item on EpiOcular™, a reconstituted three-dimensional human corneal epithelium model, were determined. Hereby, the test item was applied topically. Cytotoxicity is expressed as the reduction of mitochondrial dehydrogenase activity measured by formazan production from MTT after a 30 min exposure period and 120 min post-treatment period and compared to those of the concurrent negative controls.

The mixture of 50 µL test item per 1 mL MTT medium showed no reduction of MTT as compared to the solvent. The mixture did not turn blue/purple. Therefore, NSMTT equalled 0%.

The mixture of 50 µL test item per 1 mL Aqua dest. and per 2 mL isopropanol showed no colouring as compared to the solvent. Therefore, $NSC_{living}$ equalled 0%.

The test item showed no non-specific reduction of MTT and no relevant colouring potential after mixture with aqua dest. and with isopropanol. Therefore, no additional controls for correction of possible false-negative results were necessary.

The test item showed no irritant effects. The mean relative tissue viability (% negative control) was > 60% (96.2%).

## 8.2. Conclusion

In this study under the given conditions the test item showed no irritant effects. The test item is classified as “non-irritant“ in accordance with UN GHS “No Category” [7].

# 9. Introduction

*Serious eye damage* refers to the production of tissue damage in the eye, or serious physical decay of vision, following application of a test chemical to the anterior surface of the eye, which is not fully reversible within 21 days of application as defined by the UN GHS [7] (i.e. “Category 1”). *Eye irritation* accordingly refers to the production of changes in the eye which are fully reversible within this time range (i.e. UN GHS “Category 2”) [7]. The common method of determining eye damage/irritation is the *in vivo* Draize Rabbit Eye Test [8], [9]. In relation to animal welfare concerns, alternative methods were developed [4], [10], [11], [12], [13]. However, in the foreseeable future, no single *in vitro* eye irritation test will be able to replace the *in vivo* Draize Rabbit Eye Test to predict across the full range of irritation for different chemical classes, but strategic combinations of several alternative test methods within a tiered testing strategy might be able to fully replace the *in vivo* eye testing [14].

The EpiOcular™ Eye Irritation Test (EIT) was validated by the European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) and Cosmetics Europe from 2008 to 2013 [15], [16], [17], [18], [19]. From this validation study and its independent peer review [20] it was concluded that the EpiOcular™ EIT is able to correctly identify chemicals (both substances and mixtures) not requiring classification and labelling for eye irritation or serious eye damage according to UN GHS [7] (i.e. “No Category”), and the test method was recommended as scientifically valid for this purpose.

## 9.1. Aim of the Study

This *in vitro* method is designed to evaluate the eye hazard potential of a test chemical not requiring classification for eye irritation or serious eye damage in accordance with UN GHS [7] based on its ability to induce cytotoxicity in a reconstructed human cornea-like epithelium (RhCE) tissue construct after topical application, expressed as the reduction of mitochondrial dehydrogenase activity measured by formazan production from 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) [21].

## 9.2. Justification for the Selection of the Test System

This test uses the three-dimensional RhCE EpiOcular™ (MatTek). It consists of normal, human-derived epidermal keratinocytes and mimics the histological, morphological, biochemical and physiological properties of the human corneal epithelium. The MatTek EpiOcular™ model has been widely used as a research and testing model for many years [22].

## 9.3. Justification for the Selection of the Test Method

This *in vitro* method is recommended to identify chemicals that do not require classification for eye irritation or serious eye damage according to UN GHS (UN GHS “No Category”) [7] without further testing within a tiered testing strategy from those requiring classification and labelling (UN GHS categories 1 and 2). Therefore, it can be used for regulatory purposes as an initial step in the bottom-up approach or as one of the last steps in a top-down approach to test eye irritation/corrosion potential [14]. It is not intended to differentiate between UN GHS “Category 1” (serious eye damage) and UN GHS “Category 2” (eye irritation) which would require additional testing. Ocular irritation potential is predicted by the relative viability of the tissue after a single exposure to the test substance. Relative viability is determined by measuring the MTT dye to formazan conversion by the EpiOcular™ tissue construct after topical exposure to the test substance.

# 10. Materials and Methods

## 10.1. Characterisation of the Test Item

The identity of the test item was inspected upon delivery at the test facility (e.g. test item name, batch no. and additional data were compared with the label) based on the following specifications provided by the sponsor.

| | |
|---|---|
| Name: | PC-BC01 |
| Product: | Peptide |
| Batch No.: | 17-03 |
| Aggregate State at RT: | liquid |
| Colour: | clear and colorless |
| Storage Conditions: | < -20°C (avoid repeated freeze-thaw cycles)<br>after thawing: 2 – 8°C |
| Purity: | 95.08% |
| Expiry Date: | 29 June 2019 |
| Safety Precautions: | The routine hygienic procedures were sufficient to assure personnel health and safety. |

## 10.2. Preparation and Application of the Test Item

The test item was applied undiluted. 50 µL (83.3 µL/cm$^2$) of the test item were dispensed directly atop the EpiOcular™ tissue. The test item was spread to match the size of the tissue.

## 10.3. Controls

Controls were set up in parallel to the test item cultures in order to confirm the validity of the test.

**Negative Control**

Distilled water (Aqua dest.; Sigma, Lot No.: RNBG3520)

**Positive Control**

Methyl acetate (CAS No. 79-20-9; Merck, Lot No.: S6943111)

## 10.4. Dose Groups

| | |
|---|---|
| 1. Negative Control | 50 µL Aqua dest. |
| 2. Positive Control | 50 µL methyl acetate |
| 3. Test Item | 50 µL (undiluted) |

The test was performed on a total of 2 tissues per dose group.

## 10.5. Test System

The test was carried out with the EpiOcular™ reconstructed human cornea-line epithelium (RhCE) model (MatTek). The model consists of normal, human-derived epidermal keratinocytes which have been cultured to form a stratified, highly differentiated squamous epithelium morphologically similar to that found in a human cornea. The EpiOcular™ RhCE tissue construct consists of at least 3 viable layers of cells and a non-keratinized surface, showing a cornea-like structure analogous to that found *in vivo*.

### 10.6. Provided Materials

The EpiOcular™ tissues were provided as kits (e.g. OCL-200-EIT; MatTek), consisting of the following components relevant for this study:

1x sealed 24-well plate containing 24 inserts with EpiOcular™ tissues on agarose (Lot No.: 27080)

1x bottle EpiOcular$^{TM}$ assay medium (Lot No.: 111918ISA)

1x bottle $Ca^{2+}$/$Mg^{2+}$-free DPBS buffer (Lot No.: 082818ISA)

### 10.7. Further reagents

**MTT solution**

- MTT stock solution: 5 mg/mL MTT (VWR; Lot No.: 0977C002) in PBS (Gibco; Lot No.: 1989155)
- MTT medium: MTT stock solution was diluted 1 + 4 with DMEM-based medium (final concentration 1.0 mg/mL)

**Isopropanol** (Applichem; Lot No.: 0001365249)

### 10.8. Pre-Experiments

To check the non-specific MTT-reducing capability of the test item 50 µL of the test item were mixed per 1 mL MTT medium and incubated for 3 h in a humidified incubator at 37 ± 2 °C, 5.0% $CO_2$ / 95% air.

The mixture did not turn blue/purple. Thus, the additional test with freeze-killed tissues and the quantitative corrections were not necessary.

To check the colouring potential of the test item 50 µL of the test item were mixed per 1 mL Aqua dest. and per 2 mL isopropanol each in a 6-well plate. The water solution was incubated for at least 1 h in a humidified incubator at 37 ± 2 °C, 5.0% $CO_2$ / 95% air. The isopropanol solution was shaken on a plate shaker for 2 to 3 h. After the respective incubation period, 2 x 200 µL aliquots per test solution were transferred into a 96-well plate, using 200 µL Aqua dest. and isopropanol as respective blanks and OD was measured in a range of 570 ± 30 nm without reference wavelength in a plate spectrophotometer.

The mixture showed an $OD_{net}$ < 0.08. Thus, the additional test with viable tissues and the quantitative corrections were not necessary.

### 10.9. Experimental Procedure

Upon receipt of the EpiOcular™, the tissues were equilibrated in the 24-well shipment plate to room temperature for about 15 min. Then, the EpiOcular™ tissues were transferred into 6-well plates containing 1 mL pre-warmed assay medium per well and incubated for 1 h in a humidified incubator at 37 ± 2 °C, 5.0% $CO_2$ / 95% air. Then the inserts were transferred into new 6-well plates containing 1 mL fresh assay medium per well and pre-incubated in a humidified incubator at 37 ± 2 °C, 5.0% $CO_2$ / 95% air for 16 - 24 h.

After the overnight incubation the tissues were pre-treated with 20 µL of DPBS-buffer and incubated for 30 ± 2 min in a humidified incubator at 37 ± 2 °C, 5.0% $CO_2$ / 95% air to mimic the wet conditions of the human eye.

Afterwards, the tissues were treated with each dose group in duplicate, starting with the negative and positive control. Then the 6-well plate(s) were incubated for 30 ± 2 min at 37 ± 2 °C, 5.0% $CO_2$ / 95% air. At the end of the exposure period, the test item and control substances were removed by extensively rinsing the tissue with DPBS. Excess DPBS was removed by decanting the insert and blotting bottom with blotting paper. After rinsing, the inserts were transferred to and immersed in a prepared 12-well “post-soak plate“, containing 5 mL fresh pre-warmed assay medium per well and incubated for 12 ± 2 min at room temperature. Afterwards, the inserts were removed from the assay medium, the medium was decanted off the tissue and the tissues were blotted on blotting paper. The inserts were transferred to a new 6-well plate (post-treatment plate) containing 1 mL pre-warmed assay medium. The tissues were incubated for 120 ± 15 min at 37 ± 2 °C, 5.0% $CO_2$ / 95% air.

After this incubation period excess medium was removed by blotting bottom on absorbent paper before the inserts were transferred in a prepared 24-well “MTT assay plate” containing 0.3 mL pre-warmed MTT medium and further incubated for 3 h ± 15 min at 37 ± 2 °C, 5.0% $CO_2$ / 95% air.

After the 3 h MTT incubation period the inserts were removed, the bottom of the inserts blotted on blotting paper, and then transferred into new 24-well “extraction plates“, containing 2 mL of isopropanol. The extraction plates were sealed to inhibit isopropanol evaporation. Extraction was carried out after storage overnight in the dark at 2 - 8 °C. At the end of the extraction period the tissues were pierced and the liquid within each insert was decanted into the well from which it was taken.

Then the inserts were discarded and the extracts were mixed three times using a pipette. If any visible cell/tissue fragments were in suspension, extracts were centrifuged to eliminate the fragments and avoid further possible interference with the absorbance readings.

For each tissue 2 x 200 µL aliquots of the extract were transferred into a 96-well plate and OD was measured at 570 nm using a filter band pass of maximum ± 30 nm in a plate spectrophotometer using isopropanol as a blank.

### 10.10. Data Analysis

Ocular irritation potential of the test item was predicted from the relative mean tissue viabilities obtained after treatment compared to the negative control tissues concurrently treated with Aqua dest. The test item is considered to be irritant to the eye but it cannot be differentiated between UN GHS [7] “Category 1” or “Category 2”, if the relative tissue viability is less or equal to 60%. The test item is considered to be non-irritant in accordance with UN GHS “No Category” if relative tissue viability is higher than 60% (Table 1).

**Table 1: Prediction Model**

| **Mean tissue viability** (% negative control) | **Prediction I / NI** |
|---|---|
| ≤ 60 % | Irritant (I): No prediction can be made |
| > 60 % | Non-Irritant (NI): UN GHS “No Category” |

### 10.11. Test Acceptance Criteria

The test meets acceptance criteria if:

- mean absolute $OD_{570\ nm}$ of the negative control is > 0.8 and < 2.5
- mean relative tissue viability of the positive control is < 50%
- relative tissue viability difference of replicate tissues is < 20%.

# 11. Deviations from the Study Plan

There was the following deviation from the study plan:

**Concerning:**

Study Director, study plan, p. 2, 6

**Study Plan:**

Dr. Christine Groß

**Report:**

Dr. Helge Gehrke

**Reason:**

Project handover

This deviation did not influence the quality or integrity of the present study.

# 12. Results and Discussion

## 12.1. Results

### 12.1.1. Pre-Experiments

The mixture of 50 µL test item per 1 mL MTT medium showed no reduction of MTT as compared to the solvent. The mixture did not turn blue/purple. Therefore, NSMTT equalled 0%.

The mixture of 50 µL test item per 1 mL Aqua dest. and per 2 mL isopropanol showed no colouring as compared to the solvent. Therefore, $NSC_{living}$ equalled 0%.

The test item showed no non-specific reduction of MTT and no relevant colouring potential after mixture with aqua dest. and with isopropanol. Therefore, no additional controls for correction of possible false-negative results were necessary.

### 12.1.2. Experiment

**Table 2: Result of the Test Item PC-BC01**

| Name | Negative Control | | Positive Control | | Test Item | |
|---|---|---|---|---|---|---|
| **Replicate Tissue** | **1** | **2** | **1** | **2** | **1** | **2** |
| **Absolute $OD_{570}$** | 2.101 | 1.922 | 0.867 | 0.916 | 2.025 | 1.873 |
| | 2.131 | 1.933 | 0.862 | 0.904 | 2.074 | 1.812 |
| **Mean Absolute $OD_{570}$** | 2.022**** | | 0.887 | | 1.946 | |
| **$OD_{570}$ (Blank Corrected)** | 2.058 | 1.879 | 0.824 | 0.873 | 1.982 | 1.830 |
| | 2.088 | 1.890 | 0.819 | 0.862 | 2.031 | 1.769 |
| **Mean $OD_{570}$ of the Duplicates (Blank Corrected)** | 2.073 | 1.885 | 0.822 | 0.867 | 2.007 | 1.799 |
| **Total Mean $OD_{570}$ of the 2 Replicate Tissues (Blank Corrected)** | 1.979* | | 0.844 | | 1.903 | |
| **SD of Mean $OD_{570}$ of the Duplicates (Blank Corrected)** | 0.133 | | 0.032 | | 0.146 | |
| **Relative Tissue Viability [%]** | 104.8 | 95.2 | 41.5 | 43.8 | 101.4 | 90.9 |
| **Relative Tissue Viability Difference [%]*** ** | 9.5 | | 2.3 | | 10.5 | |
| **Mean Relative Tissue Viability [%]** | **100.0** | | **42.7**** | | **96.2** | |

* Corrected mean $OD_{570}$ of the negative control corresponds to 100% absolute tissue viability
** Mean relative tissue viability of the positive control is < 50%
*** Relative tissue viability difference of replicate tissues is < 20%
**** Mean absolute $OD_{570}$ of the negative control is ≥ 0.8 and ≤ 2.5

### 12.1.3. Test Acceptance Criteria

**Table 3: Test Acceptance Criteria**

| | Value | Cut off | pass/fail |
|---|---|---|---|
| **Mean Absolute $OD_{570\ nm}$ NC** | 2.022 | 0.8 < NC < 2.5 | pass |
| **Mean Relative Viability PC [%]** | 42.7 | < 50% | pass |
| **Max. Difference of % Viability [%]** | 10.5 | < 20% | pass |

### 12.1.4. Historical Data

**Table 4: Historical Data**

| | Mean Absolute $OD_{570\pm30nm}$ NC | Mean Relative Viability [%] PC | SD Viability [%] NC, PC, TI |
|---|---|---|---|
| **Mean** | 1.697 | 24.9 | 6.6 |
| **SD** | 0.275 | 12.9 | 7.2 |
| **Range of LCL – UCL** | 1.146 – 2.248 | 0.0 – 50.7 | 0.0 – 21.0 |
| **n** | 50 | 50 | 216 |

LCL: Lower control limit (95%, mean – 2*SD)
UCL: Upper control limit (95%, mean + 2*SD)
n: number of control values

Historical data were generated from 2014 – 2018.

## 12.2. Discussion

The potential of the test item to induce eye irritation was analysed by using the three-dimensional human corneal epithelium model EpiOcular™, consisting of normal, human-derived epidermal keratinocytes mimicking characteristics of the corneal epithelium.

In the present study PC-BC01 was applied topically to the EpiOcular™ tissue for 30 min followed by 12 min post-soaking incubation after removal of the test item. After a 120 min post-treatment period cytotoxic effects were determined via MTT reduction assay.

Ocular irritation potential of the test item was predicted from the relative mean tissue viabilities compared to the negative control tissues concurrently treated with Aqua dest.

The mixture of 50 µL test item per 1 mL MTT medium showed no reduction of MTT as compared to the solvent. The mixture did not turn blue/purple. Therefore, NSMTT equalled 0%.

The mixture of 50 µL test item per 1 mL Aqua dest. and per 2 mL isopropanol showed no colouring as compared to the solvent. Therefore, $NSC_{living}$ equalled 0%.

The test item showed no non-specific reduction of MTT and no relevant colouring potential after mixture with aqua dest. and with isopropanol. Therefore, no additional controls for correction of possible false-negative results were necessary.

The test item showed no irritant effects. The mean relative tissue viability (% negative control) was > 60% (96.2%).

The controls confirmed the validity of the study. The mean absolute $OD_{570}$ of the two negative control tissues was > 0.8 and < 2.5 (2.022). The mean relative tissue viability (% negative control) of the positive control was < 50% (42.7%). The maximum inter tissue difference of replicate tissues of all dose groups was < 20% (10.5%).

# 13. Conclusion

In this study under the given conditions the test item showed no irritant effects. The test item is classified as “non-irritant“ in accordance with UN GHS “No Category” [7].

# 14. Distribution of the Report

| | |
|---|---|
| 1 original (paper): | Sponsor |
| 1 copy (paper): | Eurofins Munich |
| 1 copy (electronic): | Sponsor |

# 15. References

## 15.1. Guidelines

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## 15.3. Internal Eurofins Munich SOPs

Standard Operating Procedure (SOP), No. 9-4-11

# 16. Appendix

## 16.1. Appendix 1: EpiOcular™ Tissue: Certificate of Analysis

# Certificate of Analysis

MatTek | IN VITRO LIFE SCIENCE LABORATORIES

**Product: EpiOcular™ Tissue**

**Lot Number:** 27080

**Part#:** OCL-200, OCL-212
Description: Reconstructed ocular tissue model containing normal human keratinocytes.
This product is for research use only. Not for use in animals, humans or diagnostic purposes.

### I. Cell source

All cells used to produce EpiOcular™ are purchased or derived from tissue obtained by MatTek Corporation from accredited institutions. In all cases, consent was obtained by these institutions from the donor or the donor's legal next of kin, for use of the cells or derivatives of the tissue for research purposes.

**Keratinocyte Strain:** 4F1188

### II. Analysis for potential biological contaminants

The cells used to produce EpiOcular™ tissue are screened for potential biological contaminants. Tests performed for each of the potential biological contaminant listed in the analysis that follows, were performed according to the test method given. The product resulted in "no detection" for the following potential biological contaminants determined by the stated test method:

**Keratinocytes:**

| | |
|---|---|
| HIV-1 virus - Oligonucleotide-directed amplification | Not detected |
| Hepatitis B virus - Oligonucleotide- directed amplification | Not detected |
| Hepatitis C virus - Oligonucleotide- directed amplification | Not detected |
| Bacteria, yeast, and other fungi - long term antibiotic, antimycotic free culture | Not detected |

### III. Analysis for tissue functionality and quality

| Test | Specification | Acceptance criteria | Result and QA Statement | |
|---|---|---|---|---|
| Tissue viability | MTT QC assay, 1 hour, n=3 | OD (540-570 nm) [1.1-3.0] | 2.081 ± 0.061 | Pass |
| Barrier function | ET-50 assay, 100 µl 0.3% Triton X-100, 3 time-points, n=2, MTT assay | ET-50 [12.2-37.5 min] | 25.01 min | Pass |
| Sterility | Long term antibiotic and antimycotic free culture | No contamination | Sterile | Pass |

Tissue viability and the barrier function tests are within the acceptable ranges and indicate appropriate formation of the mucosal barrier and a viable basal cell layer.

**Initials:** LS
**Date:** 20.11.2018

Nelson Rivas
Quality Assurance Associate

November 20, 2018
Date

CAUTION: Whereas all information above is believed to be accurate and correct, no absolute guarantee that human derived material is non-infectious can be made or is implied by this certificate of analysis. All tissues should be treated as potential pathogens. The use of protective clothing and eyeware and appropriate disposal procedures are strongly recommended.

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## 16.2. Appendix 2: Certificate of Analysis

ProCell Therapeutics, Inc.
1009 Ace-Twin Tower II, 212-30 Guro 3 Dong, Guro-gu, Seoul, Korea

# Certificate of Analysis

Material information

| | |
|---|---|
| Material code : | PC-BC01 |
| Lot No. : | 17-03 |
| Source | *E. coli* |
| Manuf. Date: | 2017.06.30 |
| Expiration date: | 2019.06.29 |
| Formulation: | 1X DPBS (Dulbecco's Phosphate Buffered Saline), 20 % glycerol, pH 7.4 |
| Storage: | < -20 ℃ (Avoid repeated freeze-thaw cycles, After thawing : 2 ~ 8 ℃ ) |

Quality control

| Items | Specifications | Results |
|---|---|---|
| Appearance | Clear and colourless solution | conformed |
| pH | pH 7.2~7.6 | pH 7.38 |
| SDS-PAGE (reduced form) | About 53 kDa single band | conformed |
| Purity (SDS-PAGE) | ≥ 90 % | 95.08% |
| Protein concentration | 20 μg/mL ± 2 | 20.1 μg/mL |
| Biological Activity ($EC_{50}$) | < 0.2 μg/mL | 0.16 μg/mL |

Certified by _____ Date 2018.06.05

HeeJe Shin

ProCell R&D Center