eurofins | BioPharma Product Testing

In vitro Skin Irritation: Human Skin Model Test (EpiDerm™) with PC-BC01

Report

Version: Final

Eurofins Munich Study No.: 187796

Sponsor:
ProCell Therapeutics
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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
und Lebensmittelsicherheit
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QFB 61-01

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)*
CV	Coefficient of variation
DMEM	Dulbecco's Modified Eagle Medium
DPBS	Dulbecco's phosphate buffered saline
e.g.	exempli gratia *(for example)*
EC	European Commission
ECVAM	European Centre for the Validation of Alternative Methods
Eurofins Munich	Eurofins BioPharma Product Testing Munich GmbH
GLP	Good Laboratory Practice
GmbH	Gesellschaft mit beschränkter Haftung *(company with limited liability)*
I	irritant
ISO	International Organization for Standardization
KT	test item treated killed tissues
KU	untreated killed tissues
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NHEK	normal human epidermal keratinocytes
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
NSMTT	non-specific reduction of MTT
OD	optical density
OECD	Organisation for Economic Co-operation and Development
PBS	phosphate buffered saline
PC	positive control
QA	Quality Assurance
QAU	Quality Assurance Unit
RhE	reconstructed human epidermis
SD	standard deviation
SDS	sodium dodecyl sulfate
SOP	Standard Operating Procedure
TKT	additional test item treated killed tissue without MTT staining
TM	test item treated living tissues
TOD_{TT}	true MTT metabolic conversion
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 #1009, Ace-Twin Tower II, 273, Digital-ro, Guro-gu, Seoul, Korea
Study Monitor:	MyeongSeop Song Biotoxtech Co., Ltd. 53 Yeongudanji-ro, Ochang-eup, Cheongwon-gu, Cheongji-si, Chungcheongbuk-do Korea
Test Facility:	Eurofins BioPharma Product Testing Munich GmbH Behringstraße 6/8 82152 Planegg Germany
Eurofins Munich Study No.:	187796
Test Item:	PC-BC01
Title:	*In vitro* Skin Irritation: Human Skin Model Test (EpiDerm™) with PC-BC01

4.3. Project Staff

Study Director:	Dr. Helge Gehrke
Team Leader Operational QA GLP/GCP/ISO:	Uwe Hamann

4.4. Schedule

Arrival of the Test Item:	24 October 2018
Study Initiation Date:	13 November 2018
Experimental Starting Date:	13 November 2018
Experimental Completion Date:	07 December 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. 439: *In Vitro* Skin Irritation: Reconstructed Human *Epidermis* Test Method, 28 July 2015 [4].

Commission Regulation (EC) No. 640/2012, L 193, Part B.46. "*In vitro* Skin Irritation: Reconstructed Human Epidermis Test Method" 06-Jul-2012 [5].

MatTek Corporation Protocol for: *In Vitro* EpiDerm™ Skin Irritation Test (EPI-200-SIT) For use with MatTek Corporation's Reconstructed Human Epidermal Model EpiDerm (EPI-200-SIT); Version 07-Nov-2014 [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.:	187796
Test Item:	PC-BC01
Title:	*In vitro* Skin Irritation: Human Skin Model Test (EpiDerm™) 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

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Date: 20 Nov 2019

7. Statement of the Quality Assurance Unit

Eurofins Munich Study No.:	187796
Test Item:	PC-BC01
Title:	*In vitro* Skin Irritation: Human Skin Model Test (EpiDerm™) 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 July 2018	25 July 2018
Audit Final Report:	18 MAR 2019	18 MAR 2019

This report reflects the raw data.

Member of the
Quality Assurance Unit:

Damla Özkan

Print Name: Damla Özkan

Date: 18 MAR 2019

8. Summary

8.1. Summary Results

In the present study the skin irritant potential of PC-BC01 was analysed. The EpiDerm™-Standard Model (EPI-200™), a reconstituted three-dimensional human epidermis model, was used as a replacement for the Draize Skin Irritation Test (OECD TG 404, [7]) to distinguish between UN GHS "Category 2" skin irritating test substances and not categorized test substances ("No Category") which may be considered as non-irritant. 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 60 min exposure and 42 h post-incubation period and compared to those of the concurrent negative controls.

The mixture of 30 µL test item per 1 mL MTT medium showed no reduction of MTT compared to the solvent. The mixture did not turn blue/purple. Therefore, NSMTT equalled 0%.

The mixture of 30 µL of the test item per 300 µl aqua dest. and per 300 µL isopropanol showed no colouring detectable by unaided eye-assessment. Therefore, NSC 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 > 50% (92.8%) after 60 min treatment and 42 h post-incubation.

8.2. Conclusion

In this study under the given conditions the test item showed no irritant effects. The relative mean tissue viability after 60 min of exposure and 42 h post-incubation was > 50%. The test item is therefore classified as "non-irritant" in accordance with UN GHS "No Category".

9. Introduction

Acute irritation is a local, reversible inflammatory response of normal living skin to direct injury caused by the application of an irritant substance for up to 4 hours. The potential to induce skin irritation is an important consideration included in procedures for the safe handling, packing and transports of chemicals [15].

Current guidelines include OECD guideline 404 [7] for acute dermal irritation and corrosion of chemicals. This guideline is based on the method described by Draize [9], and generally involves the rabbit as the experimental animal. In order to replace *in vivo* testing on skin irritation validation studies on alternative *in vitro* methods were conducted under the auspices of ECVAM [10], [11], [12], [13]. It was concluded that the modified *in vitro* EpiDerm™ Skin Irritation Test (EPI-200-SIT) showed evidence of being either a reliable and relevant stand-alone replacement test for *in vivo* skin irritation testing [14] or a partial replacement test with a testing strategy [8]. This test may be used for the hazard identification of irritant chemicals in accordance with UN GHS “Category 2”. It does not allow the classification of chemicals to the optional UN GHS “Category 3” (mild irritants). Therefore all remaining substances will not be classified, i.e. UN GHS “No Category” [15], [16], [17]. The endpoint is evaluated by MTT reduction [18].

9.1. Aim of the Study

This *in vitro* method is designed to predict and classify the skin irritation potential of a chemical by assessment of its effect on EpiDerm™, a reconstituted three-dimensional human *epidermis* model. Cytotoxicity is expressed as the reduction of mitochondrial dehydrogenase activity measured by formazan production from MTT after a 60 min exposure period.

9.2. Justification for the Selection of the Test System

This test uses the EpiDerm™ reconstructed human *epidermis* model (MatTek) which consists of normal human epidermal keratinocytes (NHEK) and therefore represents *in vitro* the target organ of the species of interest and closely mimics the biochemical and physiological properties of the upper parts of the human skin, i.e. the *epidermis*.

9.3. Justification for the Selection of the Test Method

This test method is able to detect chemicals that cause skin irritation, i.e. produce reversible damage to the skin and allows for hazard identification in accordance with UN GHS “Category 2”. Depending on the regulatory framework it can also be used to identify non-classified chemicals [4].

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) 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

Prior to treatment, all EpiDerm™ tissues were gently blotted to remove moisture. The test item was applied undiluted. 30 µL (47 µL/cm^2) of the test item were dispensed directly atop the EpiDerm™ tissue. The test item was gently spread to match size of the tissue using a bulb-headed Pasteur pipette.

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

Dulbecco's phosphate buffered saline (DPBS; Gibco, Cat. No. 14040-091, Lot No.: 1996962).

Positive Control

5% sodium dodecyl sulfate (TC-SDS-5%; MatTek, CAS No.: 151-21-3, Lot No.: 022118ISA).

10.4. Dose Groups

1.	Negative control	30 µL DPBS
2.	Positive control	30 µL 5% SDS solution
3.	Test Item	30 µL (undiluted)

The test was performed on a total of 3 tissues per dose group.

10.5. Test System

The test was carried out with the reconstituted three-dimensional human skin model EpiDerm™ (MatTek). This skin model consists of normal human epidermal keratinocytes (NHEK) which have been cultured to form a multilayered, highly differentiated model of the human *epidermis*. The NHEK are cultured on chemically modified, collagen-coated cell culture inserts (Millicell®). The EpiDerm™ *epidermis* model exhibits *in vivo*-like morphological and growth characteristics which are uniform and highly reproducible. It consists of organised basal, spinous and granular layers and a multi-layered *stratum corneum* analogous to patterns found *in vivo*.

10.6. Provided Materials

The EpiDerm™ tissues were provided as kits (e.g. EPI-200-SIT, MatTek), consisting of the following components relevant for this study:

1x sealed 24-well plate containing e.g. 24 reconstructed *epidermis* units (area: 0.63 cm^2); each reconstructed *epidermis* is attached to a cell culture insert and maintained on nutritive agar for transport (Lot No.: 28672)

2x 24-well plates

8x 6-well plates

1x bottle of assay medium (DMEM-based medium, Lot No.: 112918MSD)

1x bottle of DPBS Rinse Solution (Lot No.: 071018MSA)

1x 1 vial 5% SDS Solution (TC-SDS-5%)

25 pieces Nylon Mesh circles (8 mm diameter, 200 µm pore)

10.7. Further Reagents

MTT solution

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

Isopropanol (AppliChem; Lot No.: 0001365249)

10.8. Pre-Experiments

To check the non-specific MTT-reducing capability of the test item 30 µL of the test item were mixed per 1 mL MTT medium and incubated for 60 min at 37 ± 1 °C in the incubator.

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 30 µL of the test item were mixed per 300 µL aqua dest. and per 300 µL isopropanol each in a transparent recipient and incubated at 37 ± 1°C for 60 min.

The mixture showed no colouring detected by unaided eye-assessment. Thus, the additional test with viable tissues and the quantitative corrections were not necessary.

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.

10.9. Experimental Procedure

Upon receipt of the EpiDerm™, the tissues were inspected visually and transferred into 6-well plates containing 0.9 mL assay medium per well. The surface was dried using a sterile cotton tip and the plates were incubated in a humidified incubator at 37 ± 1 °C, 5.0% CO_2 for 60 ± 5 min. Subsequently the tissues were transferred into new wells containing 0.9 mL pre-warmed assay medium per well and were incubated for 18 ± 3 h in a humidified incubator at 37 ± 1 °C, 5.0% CO_2.

After this pre-incubation the tissues were treated with each dose group in triplicate, starting with the negative control. Start time was recorded with dosing of the first tissue and occurred sequentially for the other tissues, e.g. in one-minute intervals. After dosing of all tissues, all plates were transferred to the incubator for 35 ± 1 min. Afterwards all plates were removed from the incubator and placed under the sterile flow for the remaining time until the 60 ± 1 min incubation time of the first dosed tissue was over. Then the tissues were washed by filling and emptying the inserts 15 times with DPBS using a constant stream in about 1.5 cm distance from the tissue surface, this process also occurred sequentially, e.g. in one-minute intervals. Subsequently, the inserts were completely submerged three times in 150 mL DPBS and shaken to remove rests of the test item. Finally, the inserts were rinsed once from the inside and the outside with sterile DPBS. Excess DPBS was removed by blotting the bottom with blotting paper. The inserts were placed in prepared new 6-well plates containing 0.9 mL pre-warmed fresh assay medium per well and the tissue surface was dried using a sterile cotton tip. The plates were post-incubated at 37 ± 1 °C, 5.0% CO_2, humidified to 95%, for 24 ± 2 h. Following this incubation the tissues were transferred to new wells containing 0.9 mL fresh assay medium and incubated for additional 18 ± 2 h.

After this post-incubation period the bottom of the inserts were blotted on sterile blotting paper and the inserts were transferred in a prepared 24-well plate containing 300 µL pre-warmed MTT medium. This plate was incubated for 3 h ± 5 min at 37 ± 1 °C, 5.0% CO_2, humidified to 95%.

After the MTT incubation period, the tissues were rinsed three times with DPBS and afterwards placed on blotting paper to dry. The tissues were transferred into 12-well plates and immersed in 2 mL isopropanol, sealed to inhibit evaporation. Extraction was carried out protected from light at room temperature for at least 2 h with gentle shaking on a plate shaker.

Before using the extracts, the plate had been shaken for at least 15 min on a plate shaker and the inserts were pierced with an injection needle. The extract was pipetted up and down 3 times before 2 x 200 µL aliquots per each tissue were transferred into a 96-well plate. OD was measured at 570 nm with a filter band pass of maximum ± 30 nm without reference wavelength in a plate spectrophotometer using isopropanol as a blank.

10.10. Data Analysis

Irritant potential of the test item was predicted from the relative mean tissue viabilities compared to the negative control tissues concurrently treated with DPBS. The test item is considered to be irritant to skin in accordance with regulation EC 1272/2008 (UN GHS “Category 2”) [16], [17], if the tissue viability after exposure and post-incubation is less or equal to 50%. Further testing is required to resolve between UN GHS categories 1 and 2 and decide on the final classification of the test substance [8]. The test substance may be considered as non-irritant to skin in accordance with UN GHS “No Category” if the tissue viability after exposure and post-treatment incubation is more than 50%.

Table 1: Prediction Model

Mean Tissue Viability (% negative control)	**Prediction I / NI**
≤ 50 %	Irritant (I): UN GHS “Category 2”
> 50 %	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 three negative control tissues is ≥ 0.8 and ≤ 2.8
- mean relative tissue viability of the three positive control tissues is ≤ 20%
- standard deviation (SD) of relative tissue viability obtained from each three concurrently tested tissues is ≤ 18%.

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

The mixture of 30 µL of the test item per 300 µl aqua dest. and per 300 µL isopropanol showed no colouring detectable by unaided eye-assessment. Therefore, NSC 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**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Absolute OD_{570}	1.441	1.529	1.420	0.284	0.251	0.221	1.268	1.435	1.370
	1.445	1.546	1.420	0.294	0.260	0.225	1.277	1.444	1.389
Mean Absolute OD_{570}	1.467****			0.256			1.364		
OD_{570} (Blank Corrected)	1.400	1.488	1.379	0.244	0.210	0.181	1.227	1.394	1.329
	1.404	1.506	1.379	0.254	0.220	0.184	1.236	1.404	1.349
Mean OD_{570} of the Duplicates (Blank Corrected)	1.402	1.497	1.379	0.249	0.215	0.183	1.232	1.399	1.339
Total Mean OD_{570} of the 3 Replicate Tissues (Blank Corrected)	1.426*			0.215			1.323		
SD of Mean OD_{570} of the 3 Replicate Tissues (Blank Corrected)	0.062			0.033			0.085		
Relative Tissue Viability [%]	**98.3**	**105.0**	**96.7**	**17.4**	**15.1**	**12.8**	**86.4**	**98.1**	**93.9**
Mean Relative Tissue Viability [%]	**100.0**			**15.1****			**92.8**		
SD of Relative Tissue Viability [%]***	**4.4**			**2.3**			**5.9**		
CV [% Viabilities]	**4.4**			**15.3**			**6.4**		

* Blank-corrected mean OD_{570} of the negative control corresponds to 100% absolute tissue viability.

** Mean relative tissue viability of the three positive control tissues is ≤ 20%.

*** Standard deviation (SD) obtained from the three concurrently tested tissues is ≤ 18%.

**** The mean absolute OD_{570} of the negative control is ≥ 0.8 and ≤ 2.8.

12.1.3. Quality Criteria

Table 3: Quality Criteria

	Value	Cut off	pass/fail
Mean Absolute $OD_{570\ nm}$ NC	1.467	0.8 ≤ NC ≤ 2.8	pass
Mean Relative Viability [%] PC	15.1	≤ 20%	pass
SD Viability [%]	2.3 – 5.9	≤ 18%	pass

12.1.4. Historical Data

Table 4: Historical Data

	Mean Absolute $OD_{570±30nm}$ NC	Mean Relative Viability [%] PC	SD Viability NC, PC, TI [%]
Mean	1.808	3.8	4.1
SD	0.239	1.6	4.1
Range of LCL – UCL	1.330 – 2.287	0.5 – 7.1	0.0 – 12.2
n	47	47	223

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 2015 to 2018.

12.2. Discussion

The potential of the test item to induce skin irritation was analysed by using the three-dimensional human *epidermis* model EpiDerm™ (MatTek) comprising a reconstructed epidermis with a functional *stratum corneum*.

In the present study PC-BC01 was applied topically to the EpiDerm™ tissue for 60 min followed by a 42 h post-incubation period and immediate determination of cytotoxic effects via MTT reduction assay.

Irritant potential of the test item was predicted from the relative mean tissue viabilities obtained compared to the corresponding negative control tissues concurrently treated with DPBS.

The mixture of 30 µL test item per 1 mL MTT medium showed no reduction of MTT compared to the solvent. The mixture did not turn blue/purple. Therefore, NSMTT equalled 0%.

The mixture of 30 µL of the test item per 300 µl aqua dest. and per 300 µL isopropanol showed no colouring detectable by unaided eye-assessment. Therefore, NSC 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 > 50% (92.8%) after 60 min treatment and 42 h post-incubation.

The controls confirmed the validity of the study. The mean absolute OD_{570} of the three negative control tissues was ≥ 0.8 and ≤ 2.8 (1.467). The mean relative tissue viability (% negative control) of the positive control was ≤ 20% (15.1%). Standard deviation of viability of replicate tissues of all dose groups was ≤ 18% (2.3% - 5.9%).

13. Conclusion

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

14. Distribution of the Report

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

15. References

15.1. Guidelines

[1] 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)

[2] 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

[3] 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

[4] OECD (2015). OECD Guideline for the Testing of Chemicals No. 439: *In Vitro* Skin Irritation: Reconstructed Human *Epidermis* Test Method. 28 July 2015

[5] Commission Regulation (EC) No. 640/2012 of 06 July 2012 amending, for the purpose of its adaption to technical progress, Regulation (EC) No. 440/2008 laying down test methods pursuant to Regulation (EC) No. 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), L 193, Part B.46. "*In Vitro* Skin Irritation: Reconstructed Human Epidermis Test Method"

[6] MatTek Corporation Protocol: In Vitro EpiDerm™ Skin Irritation Test (EPI-200-SIT). For use with MatTek Corporation's Reconstructed Human Epidermal Model EpiDerm (EPI-200-SIT); Version 07 November 2014

15.2. Literature

[7] OECD (2015). OECD Guideline for the Testing of Chemicals No. 404: Acute Dermal Irritation/Corrosion. 28 July, 2015

[8] OECD (2014). Guidance Document on Integrated Approaches to Testing and Assessment for Skin Irritation/Corrosion, No. 203, Series on Testing and Assessment. 11 July 2014

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15.3. Internal Eurofins Munich SOPs

Standard Operating Procedure (SOP), No. 9-4-5

16. Appendix

16.1. Appendix 1: EpiDerm™ Skin Tissue: Certificate of Analysis

Certificate of Analysis

MatTek IN VITRO LIFE SCIENCE LABORATORIES

Product: EpiDerm™ Reconstructed Human Epidermis

Lot Number: 28672

Part#: EPI-200, EPI-212, EPI-218

Description: Reconstructed human epidermis tissue 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 EpiDerm™ 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 tissues or derivatives of the tissue for research purposes.

Keratinocyte Strain: 267S3A

II. Analysis for potential biological contaminants

The cells used to produce EpiDerm™ tissue are screened for potential biological contaminants. Tests for each potential biological contaminant listed below were performed according to the test method given. Results of "Not detected" indicate that testing for the potential biological contaminant was not observed as determined by the stated test method.

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, 4 hours, n=3	OD (540-570 nm) [1.0-3.0]	1.739 ± 0.084	Pass
Barrier function	ET-50 assay, 100 µL 1% Triton X-100, 4 time-points, n=3, MTT assay	ET-50 [4.77-8.72 hrs]	4.85 hrs	Pass
Sterility	Long term antibiotic and antimycotic free culture	No contamination	Sterile	Pass

Tissue viability and the barrier function test are within the acceptable ranges and indicate appropriate formation of the epidermal barrier, the presence of a functional stratum corneum, a viable basal cell layer, and intermediate spinous and granular layers. Results obtained with this lot conform to the requirements of the **OECD TG 431 and 439.**

Initials: SL

Date: 05.12.2018

Nelson Rivas
Quality Assurance Associate

December 5, 2018
Date

CAUTION: Whereas all information herein is believed to be 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