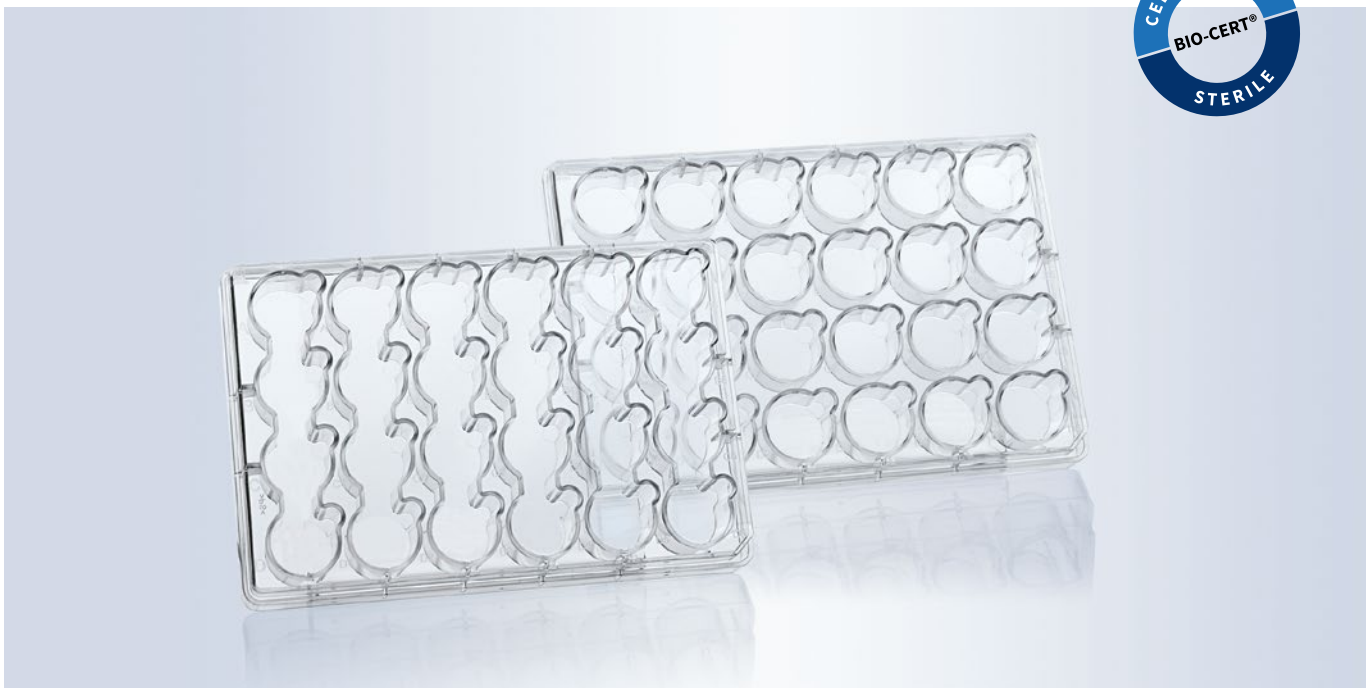
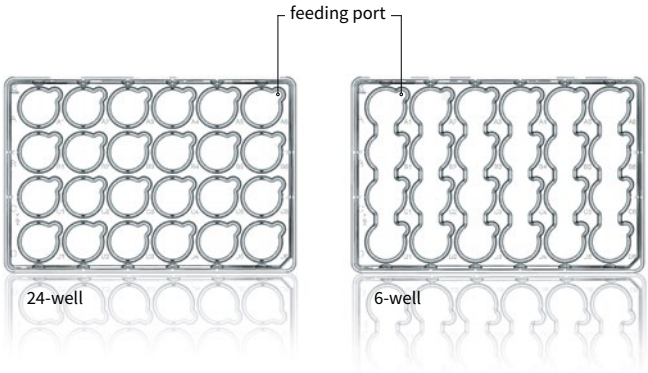
4.4 Cell culture inserts

4.4.1 Multiwell plates



- ✔ Optimal cell growth thanks to cellGrade™ plus surface
- ✔ Side well access for easier pipetting and removing cover slips
- ✔ Perfect positioning of the BRAND*plates*® insert

BRAND*plates*® multiwell plates offer better functionality than commonly available multiwell plates. Each well of the 24-well and 6-well plates has an additional extension on the edge of the well to serve as a pipette and forceps access point. This “feeding port” allows the well to be accessed even with a mounted BRAND*plates*® Insert. The additional space in the “feeding port” creates an ideal lever point for forceps to grip cover glasses without scratching them and damaging cultivated cells on the glasses.



Applications

+ Cultivation of adherent cells
+ Cultivating cells on cover glasses
+ Mounting BRAND*plates*® inserts and insert strips
+ Automated cell culture applications

Features

+ High purity, crystal-clear polystyrene
+ Conforms to ANSI/SLAS Standards 1 and 4
+ Manufactured in an ISO Class 7 cleanroom
+ Individually wrapped with lid, sterile (SAL 10^{-6})
+ Untreated or cell culture treated

User information

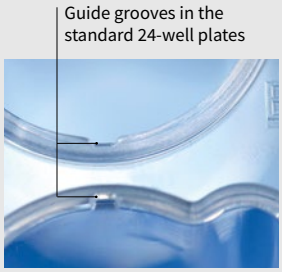
24-well standard plate

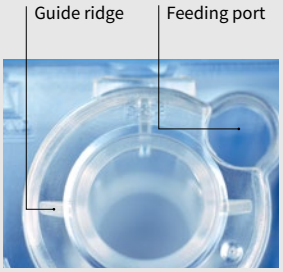
The plate includes 24 individually fillable wells that can be fitted with strips of 4 inserts and/or individual inserts

Format	24-well	6-well
Well surface [mm²] (incl. feeding port)	210	855
Working volume [ml]	3.1	10

6-well special plate

The 4 wells are all connected as one large, elongated well. This well can be fitted with a strip of 4 inserts so that all 4 of the inserts in the strip can be supplied with medium at the same time. Particularly well suited to the use of insert strips with inlet channels. Also suitable for single inserts and 2 or 3 connected inserts.





The well and insert are perfectly coordinated

The guide grooves in the support collars of the wells in the 24-well standard plate hold the guide ridges of the insert in position. This prevents the individual inserts from rotating – the feed ports on the wells remain open. At the same time, the guide ridges center the insert in the well.

Accessories

Information on accu-jet® *pro* pipetting aid and Transferpette® *S* microliter pipette is available at shop.brand.de, while information on counting chambers and centrifuge tubes is available on pages 11 and 17.

Technical information & Ordering data

24-well and 6-well

Multiwell plates

Surface	pureGrade™ S	cellGrade™ plus	pureGrade™ S	cellGrade™ plus
Wells	24	24	6	6
Working volume [ml]	3.1	3.1	8 - 10	8 - 10
Growth surface [mm²]	210	210	855	855
Lid	with lid	with lid	with lid	with lid
Pack of	10 pieces (individually wrapped)	10 pieces (individually wrapped)	10 pieces (individually wrapped)	10 pieces (individually wrapped)
Cat. No.	782880	782890	782881	782891

4.4.2 Inserts

- ✔ **Optimal cell growth thanks to cell culture treatment**
- ✔ **Add up to four inserts at once**
- ✔ **Fast, safe handling**

Cell culture inserts with microporous membranes greatly expand the range of methods that can be used in classic cell cultures.
The innovative BRAND*plates*® insert system offers a product perfectly adapted to reconstructing 3D epithelial models. The strip format ensures that the inserts sit in the well without rotation, and the 6-well plate allows for medium exchange in up to four inserts at once.

4-insert strips, divisible into up to 4 individual inserts – positioned to hang in the well

Applications

+ Epithelial cell cultures
+ Barrier analysis
+ Polarization studies
+ Epidermis models
+ Full skin models
+ Co-cultures
+ Impedance measurements

Features

+ Cell culture treated PC or PET membranes
+ Culture surface 0.6 cm^2
+ As 4x strips or individual
+ Strips divisible
+ Manufactured in an ISO Class 8 cleanroom
+ Sterile (SAL 10^{-6})

User information

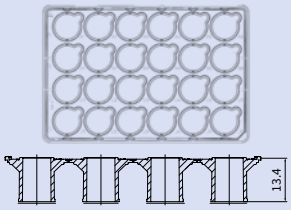
Advantages of specialized insert-plate combinations

A 3D culture of 0.6 cm^2 should be supplied with at least 1 ml of medium per day, in particular during cultivation at the air-liquid interphase. Medium must also be exchanged with the same frequency.
The BRAND insert system offers a variety of solutions to increase the provided basal volume (below the membrane) and reduce the number of medium changes.

24-well plate with 13 mm insert strip

Standard conditions for 3D cell cultures with high nutrient requirements.

Smooth-walled inserts, suitable for differentiation, transportation, co-cultivation, transmigration and cell polarity assays.

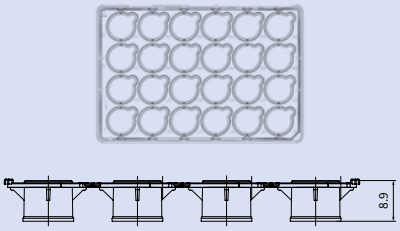


24-well plate with 9 mm insert strip

Ideal for cultures at the air-liquid interphase (ALI)

ALI cultures are supplied with 1.7 ml of medium per 24-well. This combination greatly optimizes the medium supply to ALI cultures.

Not suitable for transportation, transmigration and cell polarity assays.

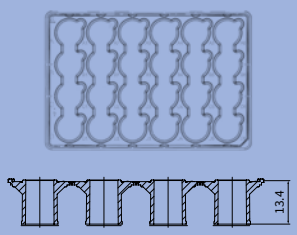


6-well plate with 13 mm insert strip

Ideal for more complex 3D cultures, such as full skin models.

When using just 2 inserts per well, each ALI culture is supplied with 1.75 ml. This means that up to 12 epithelial models can be cultivated in one plate, saving space.

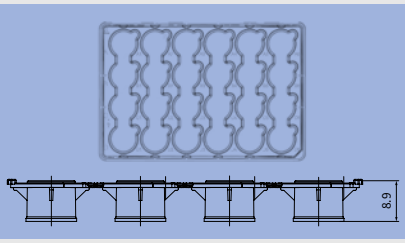
The smart 6-well design facilitates simultaneous medium exchange for all of the inserts in a series.



6-well plate with 9 mm insert strip

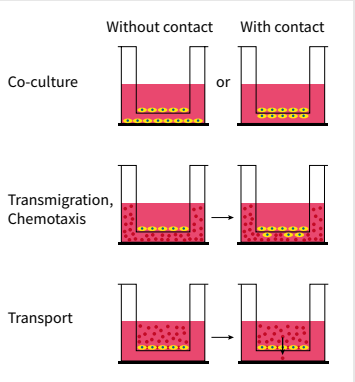
Excellent supply for cells during an air-liquid interphase culture.

When using a full strip, each culture is supplied with 2 ml of medium. Using half strips increases the basal volume to 4 ml per culture.



Co-culture:
Membranes with pore sizes of 0.4 and 1.0 µm. Use PET membranes for good cell visibility under an optical microscope. PC membranes with comparable pore sizes have a higher pore density, improving interaction between the cells than PET membranes. PC membranes, however, are not recommended for transmitted light microscopes.

Chemotaxis and transmigration:
Depending on the cell type, transmigration assays can be accomplished with pore sizes above 3.0 µm. Use PET membranes for microscopic applications.

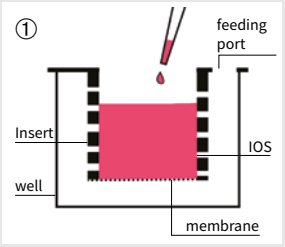


User information

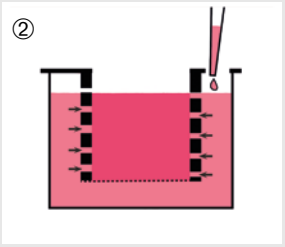
Importance and function of the Inlet Opening System (IOS)

When removing apical medium in cultures of reconstructed epithelial models, there is a danger of injuring tissue cultures with pipette tips, making the tissue culture unusable in further examinations.
The Inlet Opening System of the BRAND*plates*® Insert makes it possible to adjust the medium levels in inserts by controlling the medium level in the corresponding wells.

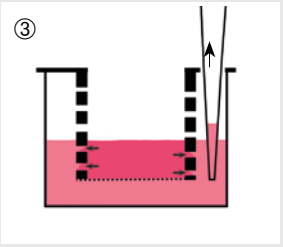
In addition to improved reliability, combining 6-well plates and inserts with IOS can reduce pipetting work for 4 inserts in fused well row from eight aspiration and filling steps to just one aspiration and one filling step. This drastically reduces the time that the cultures spend outside of the incubator, making it possible to greatly reduce the effects of temperature and pH fluctuations.



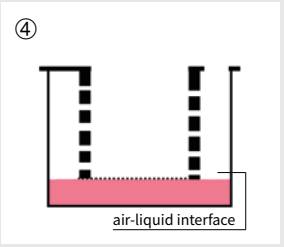
During cell seeding or applying coatings, the unique geometry of the Inlet Opening System (IOS) prevents the insert from leaking



The submersion culture is also established by adding medium to the well. The arrow indicate that the medium flows evenly into the interior of the insert.



The IOS accelerates and simplifies the medium exchange. The arrows show the direction of flow for the medium from the insert into the well where the aspiration pipette is placed.



This allows the air-liquid interface to be adjusted without the risk of tissue damage.

Possible causes for poor adhesion of cells in assay plates:

- The passage used in the cell line is too high and the cells are senescent
- The seeding density is too low
- The medium used is inadequate
- Cells are contaminated
- Cells require a specific substrate (laminin, collagen, vitronectin, fibronectin)

Recommended volumes for different culture phases of the 3D culture

	24-well	6-well	24-well	6-well
Insert height [mm]	13	13	9	9
Insert [µl] (such as coating, cell seeding)	150 - 400	150 - 400	150 - 250	150 - 250
Well: Submersion culture [ml] with added insert	1.6 - 2	8 - 10	2.2 - 2.5	9 - 10
Well: air-liquid-interphase [ml] (of basal coated membrane)	0.8	3.5	1.8	8

Membrane pore size / application examples

Pore size	Areas of application
0.4 µm	Co-culture, transport studies, secretion studies, cell polarity studies, etc.
1.0 µm	Co-culture, transport studies, secretion studies, etc.
3.0 µm	Migration studies, chemotaxis studies, metastasis experiments, etc.
8.0 µm	Migration studies, chemotaxis studies, metastasis experiments, etc. See also the construction of full-thickness skin models (www.tissue-factory.com)

Technical information & Ordering data

Insert Strips

PC membrane

Pore size	0.4 µm		1.0 µm	3.0 µm	8.0 µm
Pore density per cm²	1 x 10^8		2 x 10^7	2 x 10^6	1 x 10^5
Growth area [cm²]	0.6		0.6	0.6	0.6
Insert height [mm]	13	9	13	13	13
Pack of	12 pieces (12 strips x 4 inserts)		12 pieces (12 strips x 4 inserts)	12 pieces (12 strips x 4 inserts)	12 pieces (12 strips x 4 inserts)
Type	smooth-walled		smooth-walled	smooth-walled	smooth-walled
Cat. No.	782800	782900	782820	782840	782860
Type	IOS		IOS	IOS	IOS
Cat. No.	782801	782901	782821	782841	782861

PET membrane

Pore size	0.4 µm		8.0 µm
Pore density per cm²	1 x 10^8		1 x 10^5
Growth area [cm²]	0.6		0.6
Insert height [mm]	13	9	13
Pack of	12 pieces (12 strips x 4 inserts)		12 pieces (12 strips x 4 inserts)
Type	smooth-walled		smooth-walled
Cat. No.	782810	782910	782870
Type	IOS		IOS
Cat. No.	782811	–	782871

You need bulk packages? Five 6-well plates filled with 6 insert strips each (120 inserts) can be ordered at www.info@brand.de

Individual inserts

PC membrane

Pore size	0.4 µm	1.0 µm	3.0 µm	8.0 µm
Pore density per cm²	1 x 10^8	2 x 10^7	2 x 10^6	1 x 10^5
Growth area [cm²]	0.6	0.6	0.6	0.6
Insert height [mm]	13	13	13	13
Pack of	48 pieces	48 pieces	48 pieces	48 pieces
Type	smooth-walled	smooth-walled	smooth-walled	smooth-walled
Cat. No.	782806	782826	782846	782866

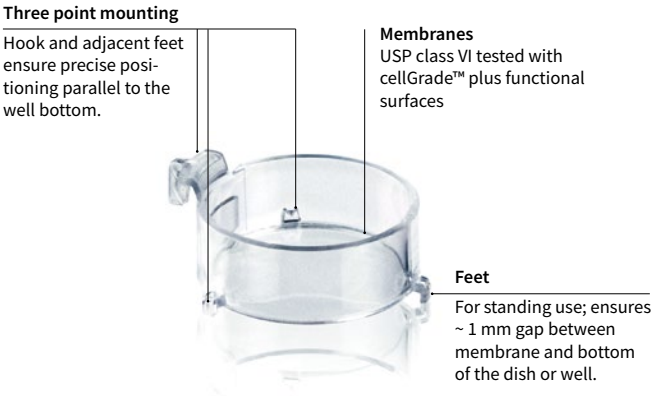
PET membrane

Pore size	0.4 µm	8.0 µm
Pore density per cm²	1 x 10^8	1 x 10^5
Growth area [cm²]	0.6	0.6
Insert height [mm]	13	13
Pack of	48 pieces	48 pieces
Type	smooth-walled	smooth-walled
Cat. No.	782816	782876

4.4.3 Insert 2in1

- ✔ Can be used standing or suspended
- ✔ Flexible and easy to use
- ✔ Cell culture treated membrane for optimal cell growth

The smart design of the BRAND Insert 2in1 allows for almost unrestricted compatibility with all ANSI/SLAS standard multiwell plates. In addition, it is the only cell culture insert of its kind that can be suspended in the well plates without additional support plates. This allows the 2in1 Insert from BRAND to provide the flexibility you need in establishing new experimental approaches.



Applications

- + Transmigration and invasion assays
- + Toxicity assessments
- + Tissue engineering
- + Barrier and transportation studies
- + Co-cultivation
- + Polarity testing
- + Cell polarization studies

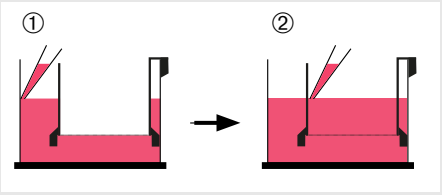
Features

- + Use in a hanging or standing position
- + Works with all common 6-, 12-, or 24-well plates
- + Surface: cellGrade™ plus
- + PC or PET membrane
- + Pore size: 0.4 µm and 8.0 µm
- + Manufactured in cleanroom ISO class 8

User information

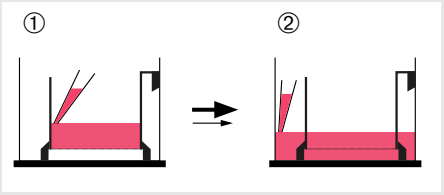
Hanging

If you use the Insert 2in1 as a hanging insert, add the medium to the multiwell plates before hanging the insert inside the wells (make sure the medium comes into contact with the membrane). Then fill the insert with medium.



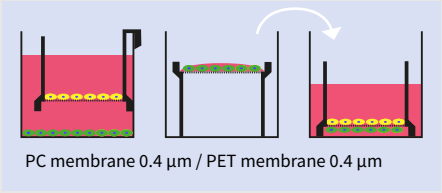
Standing

If you would like to use the Insert 2in1 as a standing insert, place the insert into the provided multiwell plate or culture dish. Add the medium to the insert and then into the well or the culture dish.



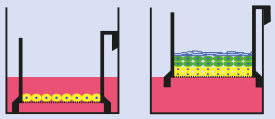
Common applications

Co-culture



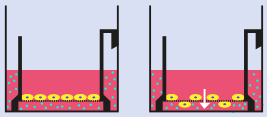
PC membrane 0.4 µm / PET membrane 0.4 µm

Air-lift culture in multiwell plate



PC membrane 0.4 µm / PC membrane 8.0 µm

Transmigration, chemotaxis

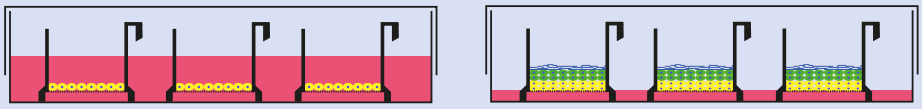


PC membrane 8.0 µm / PET membrane 8.0 µm

Transport/barrier analysis (TEER measurement), cytotoxity

PC membrane 0.4 µm

Air-lift culture in culture dish



BRAND Insert 2in1
working volume and culture area

Well	Working volume	Culture area
24-well	150 - 400 µl	0.6 cm^2
12-well	300 - 1000 µl	1.38 cm^2
6-well	800 - 3000 µl	4.83 cm^2

Increased sample volume with air-liquid interphase cultures? Switch to the BRAND*plates*® Insert System (see p. 72): same membrane, same culture surface as 24-well inserts, less time-wasting culture optimizations than when switching from other manufacturer systems.

Technical information & Ordering data

BRAND Insert 2in1

- TC treated (cellGrade™ plus) PC- and PET membranes
- Can be used with all common 24-, 12- and 6-well plates
- Use in hanging or standing position
- Individually packed or in multi-packs

Single blister

24-well Insert 2in1

PC membrane

Pore size	0.4 µm		8.0 µm	
Pore density per cm^2	1×10^8		1×10^5	
Growth area [cm^2]	0.6		0.6	
Insert height [mm]	10		10	
Type	**single blister**	**multi-pack**	**single blister**	**multi-pack**
Pack of	48 pieces	4 x 12 pieces	48 pieces	4 x 12 pieces
Cat. No.	**782700**	**782701**	**782706**	**782707**

12-well Insert 2in1

PC membrane

Pore size	0.4 µm		8.0 µm	
Pore density per cm^2	1×10^8		1×10^5	
Growth area [cm^2]	1.4		1.4	
Insert height [mm]	11		11	
Type	**single blister**	**multi-pack**	**single blister**	**multi-pack**
Pack of	48 pieces	4 x 9 pieces	48 pieces	4 x 9 pieces
Cat. No.	**782720**	**782721**	**782726**	**782727**

6-well Insert 2in1

PC membrane

Pore size	0.4 µm		8.0 µm	
Pore density per cm^2	1×10^8		1×10^5	
Growth area [cm^2]	4.8		4.8	
Insert height [mm]	11		11	
Type	**single blister**	**multi-pack**	**single blister**	**multi-pack**
Pack of	24 pieces	4 x 6 pieces	24 pieces	4 x 6 pieces
Cat. No.	**782740**	**782741**	**782746**	**782747**

Technical information & Ordering data

BRAND Insert 2in1 Multi-pack

- Quickly, conveniently open an entire pack
- Remove 3 inserts at one time
- Reduces packaging waste

Multi-pack

24-well Insert 2in1

PET membrane

Pore size	0.4 µm		8.0 µm	
Pore density per cm²	2 x 10^6		2 x 10^5	
Growth area [cm²]	0.6		0.6	
Insert height [mm]	10		10	
Type	**single blister**	**multi-pack**	**single blister**	**multi-pack**
Pack of	48 pieces	4 x 12 pieces	48 pieces	4 x 12 pieces
Cat. No.	**782710**	**782711**	**782716**	**782717**

12-well Insert 2in1

PET membrane

Pore size	0.4 µm		8.0 µm	
Pore density per cm²	2 x 10^6		2 x 10^5	
Growth area [cm²]	1.4		1.4	
Insert height [mm]	11		11	
Type	**single blister**	**multi-pack**	**single blister**	**multi-pack**
Pack of	48 pieces	4 x 9 pieces	48 pieces	4 x 9 pieces
Cat. No.	**782730**	**782731**	**782736**	**782737**

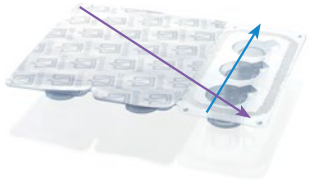
6-well Insert 2in1

PET membrane

Pore size	0.4 µm		8.0 µm	
Pore density per cm²	2 x 10^6		2 x 10^5	
Growth area [cm²]	4.8		4.8	
Insert height [mm]	11		11	
Type	**single blister**	**multi-pack**	**single blister**	**multi-pack**
Pack of	24 pieces	4 x 6 pieces	24 pieces	4 x 6 pieces
Cat. No.	**782750**	**782751**	**782756**	**782757**

To open the entire blister simply tear off the sealing paper diagonally (purple arrow).

A perforation in the sealing paper also allows opening individual rows, while the other inserts remain securely sealed (blue arrow).



Application Note

BRAND® Insert 2in1 supports the cultivation of Reconstructed Human Epidermis (RhE) used for skin corrosion tests (OECD TG 431)

Author: BRAND GMBH + CO KG

Introduction

Reconstructed Human Epidermis (RhE) is used as an alternative in vitro test system partially able to replace tests on laboratory animals and provide data that may be more predictive for humans when compared to animal testing. For these reasons 3D tissue models become more and more attractive not only for research but also in the context of regulatory hazard identification of irritant and corrosive chemicals (OECD TG 431*). However, to be used for regulatory decision making, a validated RhE must meet certain quality criteria to reliably distinguish the different hazard potentials of chemicals.
Here we show that human derived keratinocytes cultivated in the BRAND Insert 2in1 differentiate into RhE models using the standard cultivation procedure including a submerged and air-liquid interphase condition. The RhE reproducibly determines the corrosive potential of the categorized chemicals.

* OECD Test Guideline for testing chemicals 431: In vitro skin corrosion: reconstructed human epidermis (RHE) test method; 2015

Methods

Cell culture

Reconstructed human Epidermis was generated using normal human keratinocytes seeded in BRAND Insert 2in1 or cell culture inserts from another manufacturer in a density of $2*10^5$ cells/cm^2 (125.000 cells in 200 µl per insert). For submerged and air-liquid interphase (ALI) cultures both insert types were placed standing on the bottom of culture plates. BRAND Inserts featured a plasma-treated (cellGrade™ plus) polycarbonate membrane with a pore size of 0.4 µm and a culture area of approximately 0.6 cm^2. After submerged cultivation ALI-culture was initiated to induce keratinocyte differentiation into the multilayered epidermal model and finally exposed to chemicals.
MTT assay and test substance application was performed according to the SOP for epiCS® In Vitro Skin Corrosion (CellSystems®).

Test substances

For each exposure time and chemical 3 RhE models were used for in vitro skin corrosion testing. The test chemicals applied were phosphate buffered saline (PBS) (negative control), 8N KOH (positive control), 4-(Methylthio)-benzaldehyde, lactic acid and formic acid. RhE mean viability was determined for each test chemical after 3 and 60 min of exposure and normalized to the mean viability of negative controls at the corresponding time point.

Results

Morphology

The RhE models were fixed with Bouin's Solution and subsequently cryo-embedded. Following cross sections of the RhE samples were stained with hematoxylin and eosin and subjected to histological imaging. The RhE models show the typical layers of native skin with a multilayered corneal layer (stratum corneum).

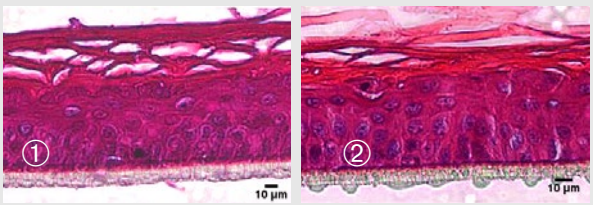


Figure 1: Hematoxylin/eosin staining of RhE models cultivated in the cell culture Insert 2in1 (①) and in an insert from another manufacturer (②). Human derived keratinocytes develop a stratified epidermis with a multilayered stratum corneum.

Barrier function test (EC50)

To determine whether the stratum corneum of RhE models cultured in different inserts developed a proper barrier function cultures were exposed to PBS and the benchmark chemical Triton X-100 for 60 min. After the exposure, RhE models were incubated in presence of MTT vital dye. Quantification of the metabolic activity was determined by measuring the optical density of the reduced MTT-dye at 570 nm wave length. Data indicates a distinct barrier function of the stratum corneum as the mean viability of the cultures was not reduced by more than 50 % at the given exposure time.

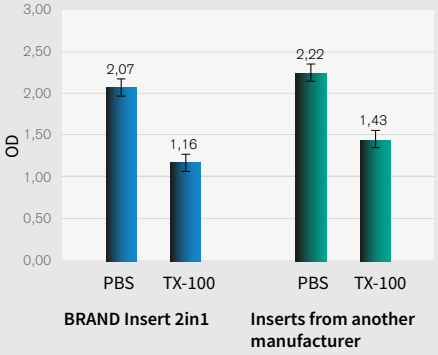


Figure 2: Viability of RhE exposed to PBS or Triton X-100 (TX-100). Data shows the optical density at 570 nm (OD) of isopropanol extracted formazan from tissue cultures.

In vitro corrosion test

First, MTT assay derived viability of RhE models was determined for 3 and 60 minutes using PBS. Measurements show that the viability of RhE models within the two insert types is comparable. However, tissue cultures from the BRAND insert 2in1 generated data with reduced standard deviations at 3 and 60 min of PBS exposure when compared to tissue culture grown in the competitor insert (table 1).

To test whether the RhE models cultivated in BRAND insert 2in1 can also be used to distinguish the corrosive potential of chemicals, RhE models were exposed to a set of classified substances. In parallel, the same chemicals were applied to RhE models cultivated in the insert from another manufacturer used in chemical hazard identification context before. The mean viability of treated RhE models was normalized to viability data of the negative control (NC).

OD negative control			
BRAND Insert 2in1		Other manufacturer	
3 min	60 min	3 min	60 min
2.92	2.21	2.76	2.38
2.95	2.22	2.82	2.45
2.96	2.44	2.48	1.79
2.94	2.45	2.47	1.75
2.96	2.52	2.08	2.23
2.99	2.51	2.06	2.13
Standard deviation OD			
0.02	0.16	0.36	0.32

Table 1: OD measurement of formazan-isopropanol extracted from RhE models exposed to PBS (NC). For each condition 6 tissues were tested. Measurements were performed in transparent flat bottom microplates using a microplate spectrophotometer at 540 -570 nm

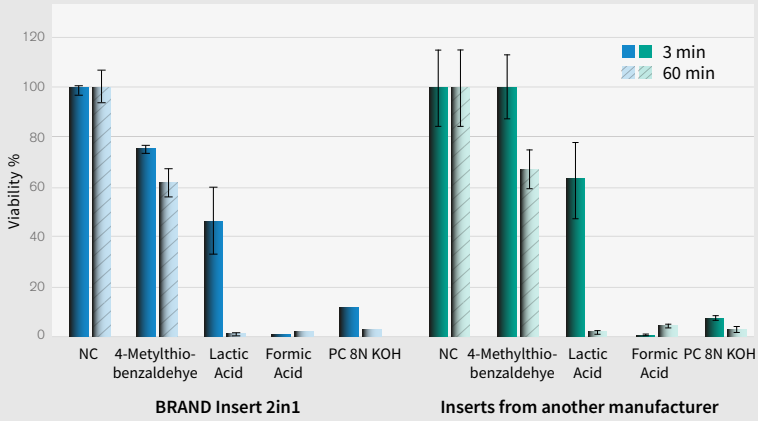


Figure 3: Comparison of corrosive potential of different chemicals. NC, negative control; PC, positive control. Data show mean viability of 3 RhE per condition with standard deviation. Viability was determined by MTT assay. Optical density of isopropanol extracted formazan was measured in microplate spectrophotometer at 540 -570 nm.

Conclusion

BRAND Inserts 2in1 are equally suitable to produce RhE as inserts from other manufacturers. This was shown by the comparison of H&E stained histological slides with the mulilayered stratified epidermis (fig.1) and the integrative growth of the keratinocytes with a functional barrier function was demonstrated by EC_{50} data (fig. 2).

Using proven chemicals for the OECD corrosion test with RhE, we could measure data comparable with inserts of another manufacturer. The BRAND Insert 2in1 is a promising tool for use in corrosion tests and a step forward to avoid animal testing and gather data much more transferable to humans than animal testing ever will be.

The presented data show that the BRAND Insert 2in1 with PC membrane and a pore size of 0.4 µm supports the differentation of normal human keratinocytes to a stratified epidermis model. Tissue models from the two inserts predicted 4-(Methylthio)-benzaldehyde as non corrosive chemical because viability is not reduced by 50 % after 3 min and 60 min of exposure when compared to NC. Formic acid is predicted to be corrosive because viability of tissue models from both inserts is reduced by more than 50% and more than 85% after exposure for 3 min and 60 min, respectively. Lactic acid is a corrosive substance of subcategory 1B/1C, which is shown by a viability higher than 50 % after 3 min and lower than 15 % after 60 min exposure, respectively. The not significant difference to the 50 % threshold within the 3 min exposure with the BRAND insert may be due to the small number of measurements.