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Reconstruction of human epidermis using BRAND*plates*® Insert System

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Reconstruction of human epidermis using BRAND*plates*® Insert System

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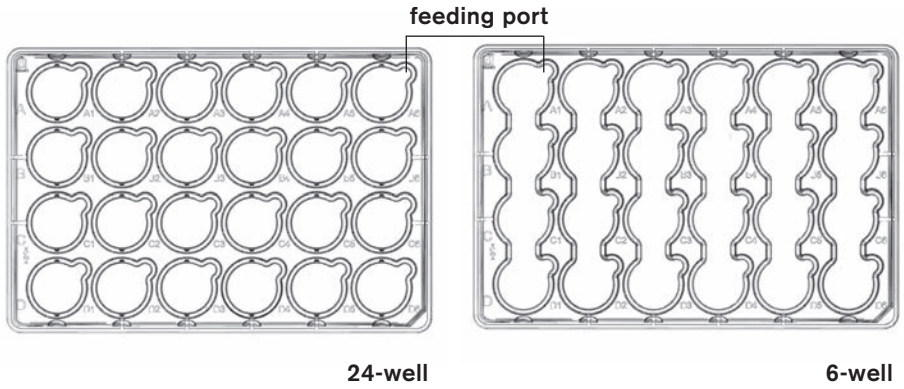
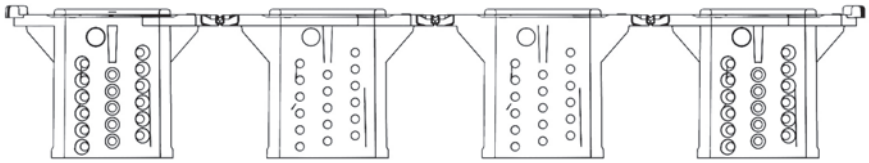
Introduction

In the past decade new cell and tissue culture technologies have been generated to comply with the European legislation that restricts animal experiments to a minimum. Particularly, the improvement of culture conditions for reconstructed human full thickness skin and epidermis equivalents based on cell culture inserts, lead to a successful commercialization of these models. Today, with the help of artificial human tissues, pharmaceutical and cosmetic industry carry out tolerance, toxicology and irritation studies daily. In spite of every progress made in terms of media compositions and supplements, setup and handling of organotypic cultures still requires a lot of time and expertise.

a) b)



c)



Hands-on time and human-induced variations in culture processes can negatively impact success in high throughput reconstruction of human tissue. To reduce sources of unintended process fluctuations and to ensure high quality and reproducibility of *in vitro* tissues, the Fraunhofer Society and BRAND GMBH + CO KG collaborated in the development of the BRAND*plates*® Insert System. This 24-well platform is specially designed to meet all requirements for a totally automated handling of insert-based tissue cultures. The carrier plates are designed in a 24-well or in a modified 6-well shape according to requirements of ANSI/SLAS standards 1 and 4 (Figure a) and b)). The corresponding BRAND*plates*® Insert Strips consist of 4 inserts in a row (Figure c) and are held in a fixed position at any time of automated handling.

6-well plate:

- Use just one or two inserts per well to extend medium change interval.
- For up to 4 inserts, medium in the well can be changed in one step (Figure b, page 3).

Inlet Opening System (IOS)

- No leaking during cell seeding or initial coating.
- Simultaneous change of medium in the well and insert.
- Setup of air-liquid-interface in one step.
- Compatible with 24- and 6-well BRAND*plates*®.

Insert:

- Divided BRAND*plates*® Insert Strips for subsequent analysis.

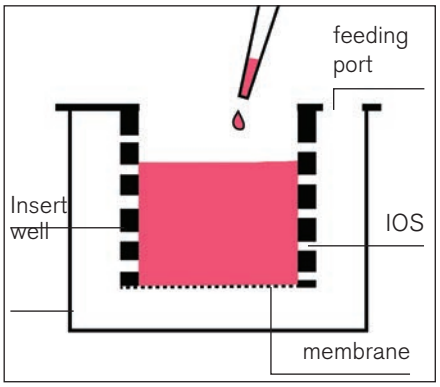
Volumes needed for different culture phases

	24-well	6-well
Insert* (e.g. coating, cell seeding)	50-400 µl	50-400 µl
Well: submerged culture*	1.6-2 ml	8-10 ml
Well: air-liquid interface** (wetted membrane)	0.8 ml	3.5 ml

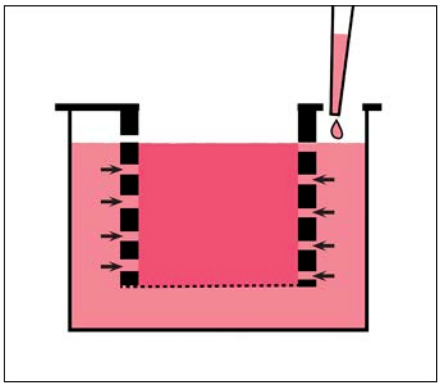
*** Submerged phase medium for inserts:**
> Keratinocyte Basal Medium 2
+ 1 % HKGS
+ 1.5 mM $CaCl_2$
+ 100 µg/ml Pen/Strep

**** Air-liquid-interphase medium:**
> submerged phase medium
+ 50 µg ascorbic acid
+ 10 ng keratinocyte growth factor

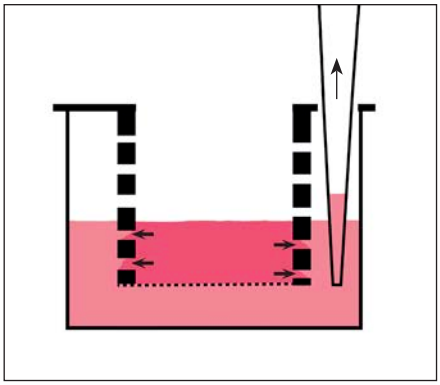
1) Coating, cell seeding



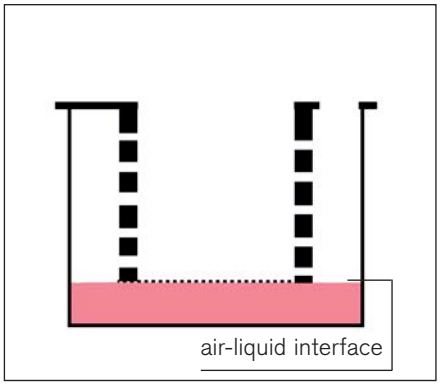
2) Medium application for submers culture



3) Medium change



4) Establish air-liquid interface

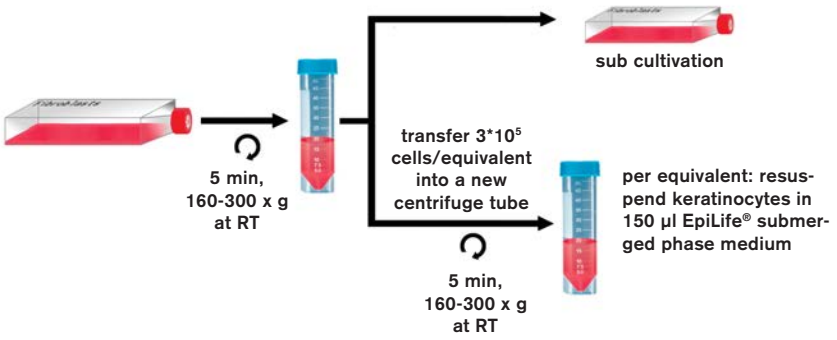


BRAND*plates*® Inserts are available with the Inlet Opening System (IOS) (patent pending, Figure c, page 3) which is dedicated to support the automated *in vitro* reconstruction of human skin. This peerless feature interconnects the medium of wells and inserts, giving the opportunity to establish the air-liquid interface without entering the inserts with pipette tips. In addition to this increase in safety for cultures, the IOS reduces the number of pipetting steps needed to change medium within the two compartments.

This user manual describes in short the reconstruction of human epidermal equivalents and provides tips for the handling of BRAND*plates*® Insert System.

I. Reconstruction of human epidermal equivalents [1]

1. Harvest of keratinocytes



Splitting of keratinocytes (passage 2)

1. Remove culture medium.
2. Wash culture with prewarmed PBS/EDTA.
3. Add 0.025 % trypsin w/v / 0.01 % w/v EDTA in PBS and incubate for 5 min at 37° C, 5 % CO_2.
4. Stop trypsin digestion by adding sub-phase medium +10 % FCS.
5. Transfer the appropriate amount of cells into a new centrifuge tube.
6. Spin down keratinocytes, aspirate serum containing medium and resuspend cells in fresh (serum free) submerged phase medium; 150 µl/insert.

Medium for sub cultivation:

Keratinocyte Basal Medium 2 (e.g. Clonetics)

+ 0.06 mM	$CaCl_2 \cdot 2H_2O$
+ 1 % human keratinocyte growth supplement (HKGS)	
0.2 ng/ml	human recombinant EGF
5 µg/ml	human recombinant Insulin
5 µg/ml	transferrin
0.5 µg/ml	epinephrine
0.2 % v/v	bovine pituitary extract
50 µM	hydrocortisone

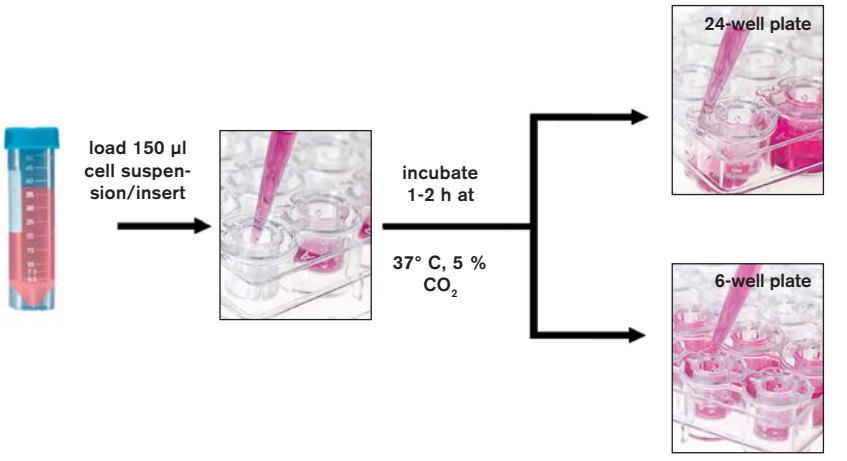
(end concentrations)

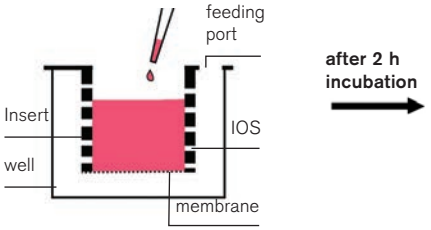
> Change medium every second day!

> Use inserts with membranes of 0.4 µm pore size!

> The use of BRAND*plates*® Inserts with Inlet Opening System (IOS) prevents culture damaging during medium application or changes and reduces number of pipetting steps needed for medium application or changes.

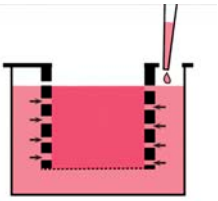
2. Seeding of keratinocytes into Insert System





after 2 h
incubation

after cell attachment apply
EpiLife® submerged phase
medium to the wells



> Check the correct position of the insert strips. Inserts should lock in place with help of plate grooves and guide ridges in 24-well plate.

> Check for air bubbles beneath the membranes.

> Slowly apply the medium to the wells by pipetting to the wall within the feeding port.

> Using one of the two central feeding ports when using the 6-well plate helps to avoid air bubbles.

Volume for submers culture in BRAND*plates*®:

> 24 well: 1.5 ml

> 6 well: 8 -10 ml

> Incubate keratinocytes for 24 h at 37°C, 5 % CO_2, 95 % humidity.

II. Working with BRAND*plates*® Insert System

Culture keratinocytes for additional 12 days at the air-liquid interface.

Medium change

	inserts/well	interval
24-well plate	1	24 h
6-well plate	4	24 h
6-well plate	2	48 h

Volumes needed for different culture phases

	24-well	6-well
Insert* (e.g. coating, cell seeding)	50-400 µl	50-400 µl
Well: submerged culture*	1.6-2 ml	8-10 ml
Well: air-liquid interface** (wetted membrane)	0.8 ml	3.5 ml

> Simultaneous handling of four inserts during different phases of post culture processing e.g. fixation, washing, staining steps etc..

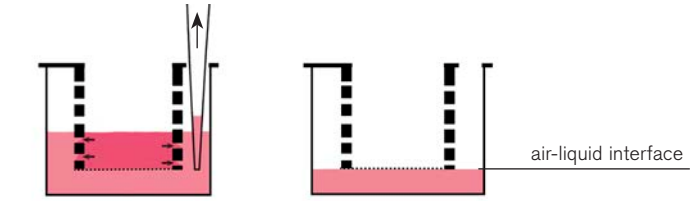
> Insert strips can be easily divided at defined break points to generate single well inserts and to perform different preparation steps on technical replicates.

Fixed and paraffin embedded reconstructed human epidermis. Sectioned perpendicular to the surface and stained with haematoxylin and eosin. Modified to the protocol of Lemper et al., 2014.

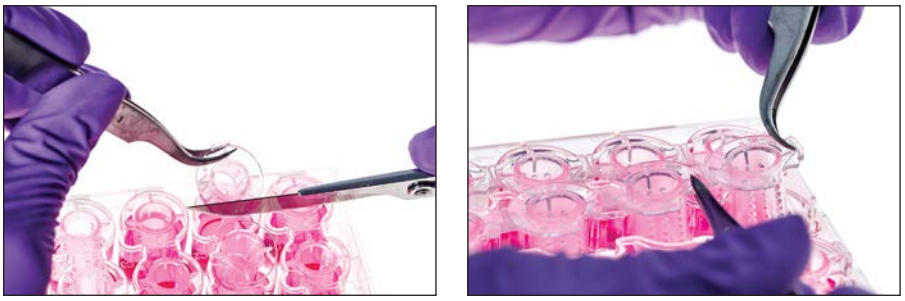
3. Establish air-liquid interface culture after 24 h incubation

Changing medium in the 6-well plate:

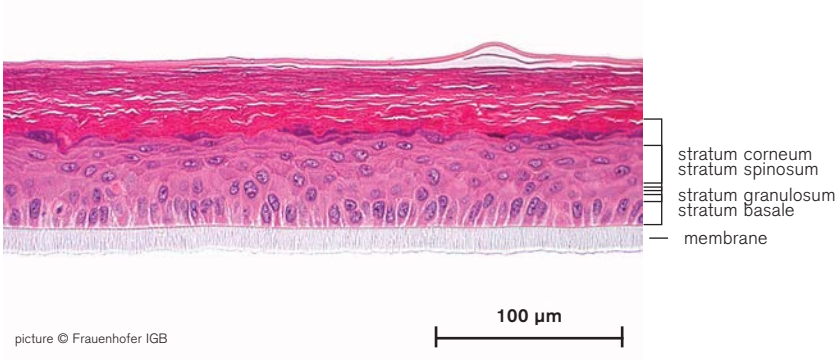
1. Exhaust medium from one well using a feeding port at the center of a strip.
2. Tilt the plate and exhaust residual medium.
3. Apply EpiLife® air-liquid interface fresh medium via one of the two central feeding ports of each well.



4. Separation of inserts for post culture processing



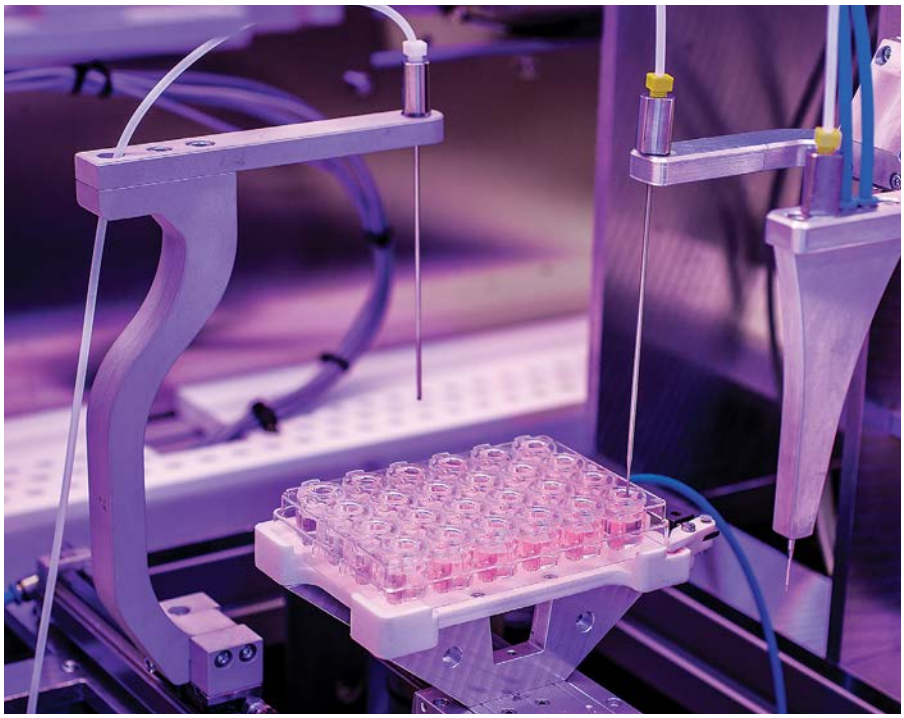
5. Representative reconstructed human epidermis cultivated in BRAND*plates*® Insert System



picture © Frauenhofer IGB

Summary

The use of the BRAND*plates*® Insert System has various advantages when compared to common cell culture inserts. The special 6-well plate utilizes a unique conjoined 24-well design to optimize centering of the well insert during the entire culture process. The geometry of inserts and plates define the so called feeding port. This extra cavity enables access to the well without shifting or rotating the inserts and disturbing the culture. The defined location of inserts and feeding ports helps to determine the position of applicators or aspirators integrated in automated processes. These attributes make the BRAND*plates*® Insert System the only 6- and 24-well culture insert platform which can be implemented totally into a robot handled cell culture.



The described properties of the 6-well plate create even more benefit when combined with BRAND*plates*® Inserts with the Inlet Opening System (IOS) (patent pending). The medium in wells and inserts is interconnected by the IOS, so that it is possible to control medium level in both compartments simultaneously resulting in just 6 instead of 48 pipetting steps during medium exchange and when an air-liquid interface needs to be established.

The fusion of four inserts into one insert strip also provides remarkable advantages. Whenever inserts have to be moved, it is easy to grip one strip and transfer the four inserts in one step, e.g. transferring inserts from well to well during fixation, dehydration, washing or staining steps.

References:

[1] Lemper et al., 2014: Practical Problems Encountered during the Cultivation of an Open-Source Reconstructed Human Epidermis Model on a Polycarbonate Membrane and Protein Quantification. Skin Pharmacol Physiol 2014; 27:106-122

[2] Poumay Y, Dupont F, Marcoux S, Leclercq-Smekens M, Hérin M, Coquette A: A simple reconstructed human epidermis: preparation of the culture model and utilization in in vitro studies. Arch Dermatol Res 2004; 296:203-211

Ordering Data

BRAND*plates*® Insert Strips**

Insert strips, smooth-walled or with inlet channels (Inlet Opening System*)

PS. cellGrade™ plus surface, sterile. Strips of 4 inserts (divisible).

Description	Pore size µm	Pack of	PC membrane Cat. No.	PET membrane Cat. No.
smooth-walled	0.4	12 (individually wrapped)	**782800**	**782810**
with Inlet Opening System	0.4	12 (individually wrapped)	**782801**	**782811**

BRAND*plates*® Insert System**

6-well plates filled with 6 insert strips

PS. cellGrade™ plus surface, sterile. Insert strips, smooth-walled or with inlet channels (Inlet Opening System*). With lid with condensation rings.

Description	Pore size µm	Pack of plates with lid	PC membrane Cat. No.	PET membrane Cat. No.
smooth-walled	0.4	5 (30 insert strips)	**782802**	**782812**
with Inlet Opening System	0.4	5 (30 insert strips)	**782803**	**782813**

* patent pending
** additional Insert Plates, Strips and System products available. For more information visit www.brandtech.com.

For product information in the USA & Canada contact Brandtech® Scientific, Inc. at 888-522-2726

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