

ThinCert™

6, 12 and 24 Well Cell Culture Inserts for Multiwell Plates

For advanced cell and tissue culture applications, Greiner Bio-One offers an extensive family of membrane supports - ThinCert™. Combining 6 different membrane specifications (pore size and density) in geometries to fit 6, 12 and 24 well plates, the ThinCert™ cell culture inserts are suitable for a wide range of applications including transport, secretion and diffusion studies, migrational experiments, cytotoxicity testing, co-cultures, trans epithelial electric resistance (TEER) measurements, as well as primary cell culture. ThinCert™ cell culture inserts are compatible with standard CELLSTAR® multiwell plates from Greiner Bio-One, and are pre-packed together with the requisite number of plates. The automated production process includes double optical control of each insert produced, ensuring that any biological contamination is avoided. The sterility of the single blister-packed inserts and multiwell plates is ensured by irradiation.

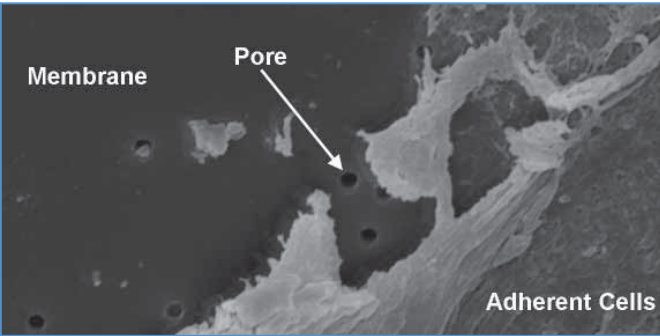


Figure 1: Electron micrograph of human osteosarcoma cells on ThinCert™ membrane. Courtesy of the Department of Oral and Maxillar Facial Surgery, University Hospital Freiburg.

ThinCert™ cell culture inserts are produced from high-grade clear polystyrene housings, and polyethylene terephthalate (PET) capillary pore membranes. Both materials, polystyrene and PET, are USP class VI certified and cell culture compatible. The coupling between the housing and the membrane is achieved using an automated process which produces an extremely strong and robust seal without compromising or weakening the membrane in any way. The membranes undergo a physical surface treatment to optimise cellular adherence and growth characteristics (Fig. 2). All the capillary pores in a membrane exhibit a high degree of uniformity in diameter. This uniformity ensures reliable and consistent exchange rates between the two compartments and thus provides reproducibility when conducting multiple experiments.

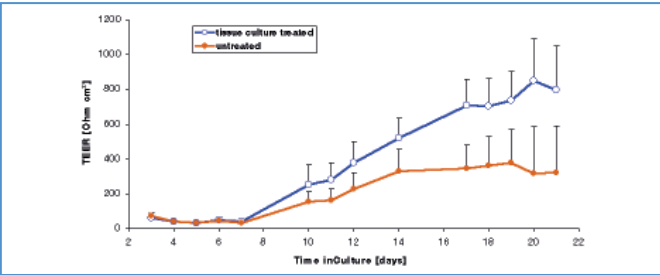


Figure 2: Different growth of bronchial epithelial cells on non-treated vs. TC-treated membranes, measured by TEER (Trans Epithelial Electric Resistance). Courtesy of the Department of Biopharmaceutics and Pharmaceutical Technology, University of Saarbruecken.

For light or electron microscopy applications, the membranes can be easily detached from the housing using a scalpel, and once detached, the membrane stays flat and does not curl up, simplifying further manual working steps and avoiding loss of cells. Due to a high chemical resistance to solvents (→ p. A I 8) a broad spectrum of cell fixation protocols can be performed.

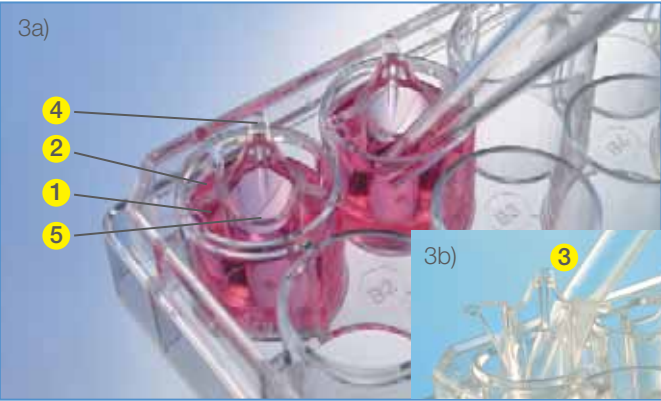


Figure 3a: ThinCert™ cell culture inserts
Figure 3b: "Self-lift" geometry of ThinCert™ cell culture inserts



Figure 4: ThinCert™ cell culture inserts packaging

The specific hanging geometry design of the ThinCert™ cell culture inserts ensures that there is always a gap between the membrane support and the bottom of the well. This avoids damage to the cells growing in the lower compartment. In addition the spacers (Fig. 3a → 1) prevent capillary suction between the side of the well and the ThinCert™ housing. Consequently component exchange between compartments can only take place through the membrane pores. The ThinCert™ cell culture inserts sit in an eccentric position within the well (Fig. 3a → 2). This specific design gives rise to the so called "self-lift" function, with the insert sliding easily upwards when the pipette is inserted into the lower compartment and gliding back into its original position after the pipette has been withdrawn (Fig. 3b → 3). The largest of three pipetting openings is located opposite of the small conical foot (Fig. 3a → 4). This allows for very convenient pipetting of media or supplements even with the ThinCert™ remaining in the well.

The scalloped rims (Fig. 3a → 5) of the ThinCert™ cell culture inserts allow for flatter pipetting angles. This helps to minimise the risk of contamination as the hand of the user does not remain above the open cell culture. Additionally, the scallops significantly enhance the freedom of movement when pipetting and enhance gas exchange during cultivation.

The sales carton can be used as a donor box (Fig. 4). The required number of ThinCert™ cell culture inserts and CELLSTAR® cell culture plates may conveniently be removed from the donor box, whereas the remaining parts may safely be stored in it.

Which Membrane to use?

General Aspects:

- Small pore sizes (0.4 and 1 µm in diameter) for co-cultivation as well as for transportation, secretion, and diffusion studies
- Larger pore sizes (3 and 8 µm in diameter) for migration and invasion studies
- Transparent membranes (in general low pore density) suitable for light and electron microscopy
- Translucent membranes (in general high pore density) suitable for electron microscopy, TEER, and transport assays

For detailed application protocols and a comprehensive bibliography visit www.gbo.com/bioscience/thincert



Further information on ThinCert™

- **Forum No. 8: ThinCert™ cell culture products – Overview** (F073 017)
- **ThinCert™ Migration Assay** (F073 115)
- **ThinCert™ Invasion Assay** (F073 114)
- **Application Note “Immunocytochemistry”** (F073 100)
- **Application Note “Skin models”** (F074 062)
- **Application Note “Co-culture”** (F074 059)
- **Application Note “TEER and impedance measurements”** (F073 037)

	0.4 µm transparent	0.4 µm translucent	1.0 µm transparent	3.0 µm transparent	3.0 µm translucent	8.0 µm translucent
Light microscopy	+	-	+	+	-	+
Electron microscopy	+	+	+	+	+	+
Fluorescence microscopy	+	+	+	+	+	+
Immunocytochemistry	+	+	+	+	+	+
Trans epithelial electric resistance (TEER)	+	+	+	+	+	+
Transport and uptake studies	-	+	+	+	+	+
Co-cultivation	+	+	+	-	-	-
Cell retention	+	+	+	-	-	-
Migration/chemotaxis/metastasis	-	-	-	+	+	+

+* very suitable

+ suitable

- not suitable

Table 1: Suitability chart of the different ThinCert™ membrane types

2 HTS-
Microplates3 Immunology/
HLA4 Microbiology/
Bacteriology5 Tubes/Multi-
Purpose Beakers6 Liquid
Handling7 Molecular
Biology8 Protein
Crystallisation

9 Separation

10 Biochips/
Microfluidics11 Cryo-
Techniques12 Lids/Sealers/
CapMats13 Reaction Tubes/
Analyser Cups

14 Accessories



Free of detectable
DNase, RNase,
human DNA
non-pyrogenic

non-
cytotoxic

ThinCert™ Cell Culture Inserts 6 Well, 12 Well

Cell Culture Multiwell Plates p.1 | 11

Pre-configured Multiwell Plates with ThinCert™
Cell Culture Inserts available on request

Features

- Stable clear polystyrene housing
- Hanging geometry
- Sealed PET capillary pore membrane
- Single, sterile blister packing
- Improved cell adhesion through physical surface treatment
- Simplified pipetting due to self-lift geometry
- Enhanced pipetting access and gas exchange



6 w e l l	Cat.-No.	657 640	657 641	657 610	657 630	657 631	657 638
	Pore size [µm]	0.4	0.4	1.0	3.0	3.0	8.0
	Pore density [cm ⁻²]	1 x 10 ⁸	2 x 10 ⁶	2 x 10 ⁶	0.6 x 10 ⁶	2 x 10 ⁶	0.15 x 10 ⁶
	Optical membrane properties	translucent	transparent	transparent	transparent	translucent	translucent
	Culture surface [mm ²]	452.4	452.4	452.4	452.4	452.4	452.4
	Height (overall) [mm]	16.25	16.25	16.25	16.25	16.25	16.25
	Inner/Outer diameter (top) [mm]	24.85/27.85	24.85/27.85	24.85/27.85	24.85/27.85	24.85/27.85	24.85/27.85
	Working volume ThinCert™ [ml]	1.0 – 3.6	1.0 – 3.6	1.0 – 3.6	1.0 – 3.6	1.0 – 3.6	1.0 – 3.6
	Working volume well [ml]	2.0 – 4.15	2.0 – 4.15	2.0 – 4.15	2.0 – 4.15	2.0 – 4.15	2.0 – 4.15
	TC surface treatment/Sterile	+/+	+/+	+/+	+/+	+/+	+/+
	Multiwell plates/box	4	4	4	4	4	4
	ThinCert™ inserts/box	24	24	24	24	24	24



12 w e l l	Cat.-No.	665 640	665 641	665 610	665 630	665 631	665 638
	Pore size [µm]	0.4	0.4	1.0	3.0	3.0	8.0
	Pore density [cm ⁻²]	1 x 10 ⁸	2 x 10 ⁶	2 x 10 ⁶	0.6 x 10 ⁶	2 x 10 ⁶	0.15 x 10 ⁶
	Optical membrane properties	translucent	transparent	transparent	transparent	translucent	translucent
	Culture surface [mm ²]	113.1	113.1	113.1	113.1	113.1	113.1
	Height (overall) [mm]	16.25	16.25	16.25	16.25	16.25	16.25
	Inner/Outer diameter (top) [mm]	13.85/15.85	13.85/15.85	13.85/15.85	13.85/15.85	13.85/15.85	13.85/15.85
	Working volume ThinCert™ [ml]	0.3 – 1.0	0.3 – 1.0	0.3 – 1.0	0.3 – 1.0	0.3 – 1.0	0.3 – 1.0
	Working volume well [ml]	1.0 – 2.0	1.0 – 2.0	1.0 – 2.0	1.0 – 2.0	1.0 – 2.0	1.0 – 2.0
	TC surface treatment/Sterile	+/+	+/+	+/+	+/+	+/+	+/+
	Multiwell plates/box	4	4	4	4	4	4
	ThinCert™ inserts/box	48	48	48	48	48	48



ThinCert™ Cell Culture Inserts 24 Well

- ▶ Cell Culture Multiwell Plates p. 1 | 11
- ▶ Pre-configured Multiwell Plates with ThinCert™ Cell Culture Inserts available on request

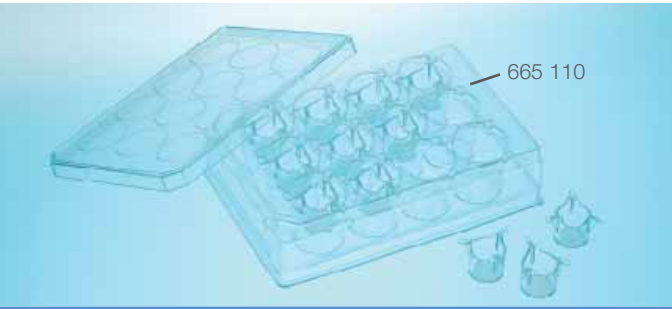
- Features**
- Stable clear polystyrene housing
 - Hanging geometry
 - Sealed PET capillary pore membrane
 - Single, sterile blister packing
 - Improved cell adhesion through physical surface treatment
 - Simplified pipetting due to self-lift geometry
 - Enhanced pipetting access and gas exchange

Free of detectable
DNase, RNase,
human DNA
non-pyrogenic

non-
cytotoxic

24 Well							
	Cat.-No.	662 640	662 641	662 610	662 630	662 631	662 638
	Pore size [µm]	0.4	0.4	1.0	3.0	3.0	8.0
	Pore density [cm ²]	1 x 10 ⁸	2 x 10 ⁶	2 x 10 ⁶	0.6 x 10 ⁸	2 x 10 ⁶	0.15 x 10 ⁶
	Optical membrane properties	translucent	transparent	transparent	transparent	translucent	translucent
	Culture surface [mm ²]	33.6	33.6	33.6	33.6	33.6	33.6
	Height (overall) [mm]	16.25	16.25	16.25	16.25	16.25	16.25
	Inner/Outer diameter (top) [mm]	8.4/10.4	8.4/10.4	8.4/10.4	8.4/10.4	8.4/10.4	8.4/10.4
	Working volume ThinCert™ [ml]	0.1 – 0.35	0.1 – 0.35	0.1 – 0.35	0.1 – 0.35	0.1 – 0.35	0.1 – 0.35
	Working volume well [ml]	0.4 – 1.2	0.4 – 1.2	0.4 – 1.2	0.4 – 1.2	0.4 – 1.2	0.4 – 1.2
	TC surface treatment/Sterile	+/+	+/+	+/+	+/+	+/+	+/+
	Multiwell plates/box	2	2	2	2	2	2
	ThinCert™ inserts/box	48	48	48	48	48	48

ThinCert™Plate



ThinCert™Plate

Cell Culture Multiwell Plates p. 1 | 11

- Optimised for use with ThinCert™ cell culture inserts
- Deep wells for an increased volume of medium in air-lift culture
- Notches for fixed insert position
- Available in 6 and 12 well format

ThinCert™Plate

The in-vitro reconstruction of many types of epithelia, such as skin, cornea and airway epithelium, requires that the cells used differentiate at the air-liquid-interphase (air-lift culture). Therefore, the tissue is cultivated in permeable membrane supports (cell culture inserts), with the cell culture medium nourishing the cells from below the membrane and the air exerting its influence from above (Fig. 1b). If cell culture inserts and conventional cell culture plates are utilised in the air-lift culture, the available volume of culture medium is severely limited (Fig. 1b). This reduced volume results in an elevated nutrient consumption rate from the lower compartment and an increased number of medium exchanges. With the ThinCert™Plate, Greiner Bio-One offers an innovative solution for the air-lift culture with ThinCert™ cell culture inserts. The plate is available in 6 and 12 well format. Its deep wells allow a larger volume of medium to be available to the air-lift culture (Fig. 1c). As a consequence, the frequency of medium exchanges may be reduced to one medium change per week. Furthermore, the reduced frequency of medium changes ensures that the medium conditioning lasts longer and an improved tissue quality is achieved. The ThinCert™Plate design consists of notches for fixing the position of the inserted ThinCert™ cell culture inserts (Fig. 2). This ensures a stable and predictable position of the inserts during the entire process of cell culture. With ThinCert™ cell culture inserts and the optimised ThinCert™Plate Greiner Bio-One provides the professional answer to the challenges of tissue reconstruction *in vitro*.



Figure 2: ThinCert™Plate: Notches (arrows) secure the position of the ThinCert™ cell culture inserts during cell culture.

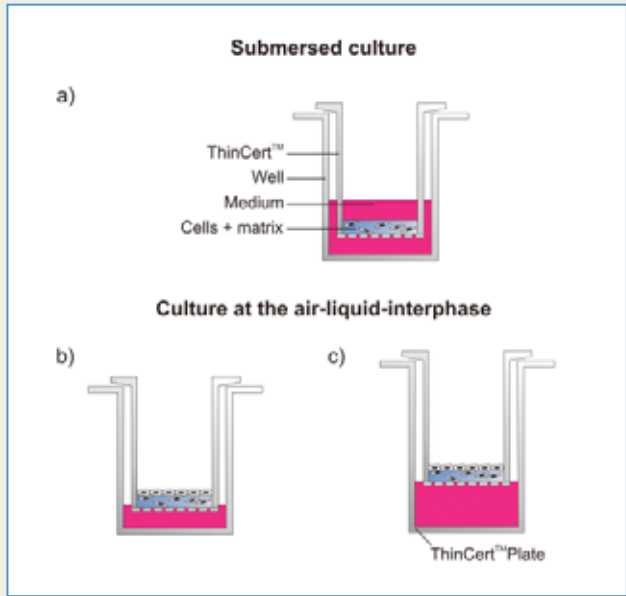


Figure 1: Reconstruction of a full thickness skin *in vitro*. a) A permeable membrane support carries fibroblasts in an extracellular matrix (e.g. Collagen). This dermis equivalent is cultivated under submersed culture conditions. b) Keratinocytes are superimposed upon the dermis equivalent and differentiated at the air-liquid-interphase (air-lift culture). If performed in a regular cell culture plate only a very limited medium volume is available for the air-lift culture. c) The ThinCert™Plate enables the air-lift culture to access an increased medium volume thereby reducing the frequency of medium exchanges. This allows the cells to stay longer in their conditioned environment which may lead to an increase in tissue quality.

Cat.-No.	657 110	665 110
Well format	6 well	12 well
Total volume per well [ml]	30	10
Working volume in air-lift culture [ml]	20	4
Sterile	+	+
Lid	+))	+))
Quantity per bag/case	1/50	1/60

+) with condensation rings