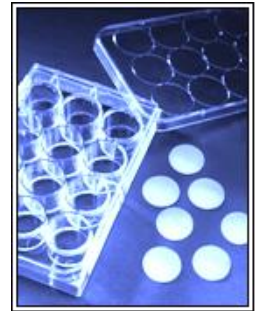


## Collagen Cell Carrier

### Product Description

CCC scaffolds are sterile, compact membranes of pure, non-cross-linked bovine collagen type I for the cultivation of cells on their surface. For use in cell culture plates, specially formatted discs are available. The 50 mm x 50 mm format can be cut by the customer according to his requirements with sterilized scissors or a scalpel.

The CCC is delivered dry and individually packed. For cell loading it can be attached reversibly to the bottom of a cell culture-treated well plate without the need of any auxiliary compound. The attachment protocol below should be observed to ensure proper adhesion of the CCC to the well.



### Applications

The CCC is a robust, sterile **Collagen Cell Carrier** for the growth and differentiation of various cell types, representing a close-to-natural extracellular matrix analogue for use in conventional cell culture-treated well plates. It is standardized due to industrial production methods and also allows the combination with additional matrix molecules and growth factors. It is best suited for cultivation of adherent primary cells, stem cells and cell lines. Also allowing directed differentiation it represents an excellent scaffold for complex tissues and tissue engineering. Additionally, the high mechanical strength of the collagen membrane permits the easy and sterile translocation of the intact cell-scaffold complex e.g. for transplantation experiments or histological analyses.

#### *Passaging*

For cell passaging or preparation of cell suspensions (e.g. for flow cytometry) standard trypsinization procedures can be used to detach adherent cells from the CCC.

#### *Immunofluorescence*

The ultra-thin and translucent membrane exhibits a very low autofluorescence that makes the scaffold applicable for fluorescent imaging of cultured cells.

The labelling procedure can be carried out directly in the well followed by transferring the intact, cell-seeded CCC for direct embedding on the slide or in the resin.

#### *Histological analysis*

Histological analysis requiring fixation of cells on the CCC can be performed by standard fixation protocols using paraformaldehyde, glutaraldehyde, or methanol.

The CCC can be frozen or embedded into paraffin or epon and sliced with a cryostat or microtome, respectively. Therefore, the scaffold is also suitable for electron microscopic investigations.

#### *Implantation*

CCCs exhibit excellent biocompatibility in vivo. In various experiments resorption was observed several weeks post implantation, depending on the target organ, without notable immunoreaction.

#### *Metabolic analysis of cells with colorimetric methods*

Cell vitality and growth on the CCC can be monitored by colorimetric methods (e.g. WST-1, MTT test) according to the manufacturer's recommendations.

## Storage

The originally packed Collagen Cell Carrier should be stored between +15°C and +25°C in closed packaging in a dry and dark place.

Storage life: 24 months

**PLEASE NOTE: COLLAGEN CELL CARRIER (CCC) PRODUCTS ARE INTENDED FOR RESEARCH USE ONLY. THEY ARE NEITHER INTENDED FOR HUMAN NOR ANIMAL DIAGNOSTIC, THERAPEUTIC USE NOR ANY OTHER CLINICAL USES.**

### **BEFORE STARTING, PLEASE NOTE:**

When working with CCCs use appropriate cell culture-treated plates, media, and reagents as well as aseptic techniques and ensure adequate growth environments.

All liquids should be pre-warmed to 37°C.

Always add liquids gently by tapping the sidewall of the well with the tip of the pipette; gently fill liquids into the well along the sidewall.

After attachment to the bottom of the well plate avoid touching the CCC membrane until its translocation is intended.

If the CCC is used as a barrier to shield populations of cells from another, make sure not to pierce the membrane with forceps or pipettes.

After detachment the intact CCC-cell complex can be transferred from the well to a plain support (e.g. a glass slide). Wet all surfaces to facilitate gliding of the flat cell-seeded CCC.

## **Viscofan BioEngineering**

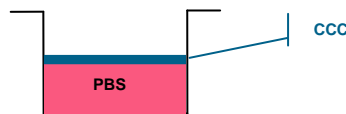
A Business Unit of Naturin Viscofan GmbH  
Badeniastraße 13  
69469 Weinheim  
Germany

Tel.: +49 (0)6201 86-358  
Fax: +49 (0) 6201 86-226  
Email: [sales@bio.viscofan.com](mailto:sales@bio.viscofan.com)  
[www.viscofan-bioengineering.com](http://www.viscofan-bioengineering.com)

### Attachment of the CCC to the well bottom and cell seeding

	Ø 34mm (6-well)	Ø 14mm (24-well)	Ø 10mm(48-well)
Volume of PBS	1000µl	250µl	150µl

1. Preload each well with the given volume of PBS (pH 7.3 without Ca<sup>2+</sup> / Mg<sup>2+</sup>)  
Do not exceed these volumes.
2. Take a CCC with sterilized forceps out of the blister and place it onto the liquid.  
Do not submerge.



3. Incubate for 15 to 30 minutes at room temperature.
4. Remove the remaining PBS.  
Assure that the CCC is flatly positioned on the bottom of the well.
5. Let the plate stand in the operating laminar flow hood overnight.  
The dry CCC is slightly opaque and will become transparent again after wetting.
6. Prior to cell seeding, equilibrate the CCC by incubation with an appropriate volume of the desired pre-warmed culture medium for at least 10 min at 37°C.  
For cell types known to be sensitive to phosphate buffers, the CCC may be washed with ddH<sub>2</sub>O before incubation with medium.
7. Remove the medium just prior to seeding cells onto the CCC.

### Detachment of cell loaded CCC from the well plate

1. Remove the medium from the well except for a small volume and wet the inner side wall of the well with some medium.
2. Optionally, to loosen the CCC, circuit it once with the tip of a pointed forceps.
3. Grasp the CCC-cell-complex at one side with the forceps and remove it from the bottom by pulling gently to the opposite well side in a steep angle.  
In case it sticks rather tightly, remove small areas of the CCC boundary at several sides before pulling.
4. Briefly re-position the scaffold loosely on the well bottom and immediately pull it flatly up the wetted sidewall.
5. Instantly, place a glass slide or a similar support close to the well, wet it with any buffer, water or medium and carefully pull the CCC directly from the side wall onto the support.