USER GUIDE

ATTACHMENT & DETACHMENT OF CCC

Collagen Cell Carrier®
for cell/tissue transfer

Attachment to and detachment of the Collagen Cell Carrier® (CCC) from the well bottom

The CCC is a native cell matrix designed as a mobile cell and tissue carrier. As such, the cell-seeded CCC may be removed from the well and transferred to various subsequent processes (e.g. implantation studies, embedding for histological analyses etc.).

Before cell seeding, please follow the protocol on page 2 to (reversibly) attach the CCC to the well bottom.

For subsequent removal and transfer of the cell-seeded CCC, please follow the CCC detachment protocol on page 3.

General precautions

When working with the CCC please use appropriate cell culture plastics, media and reagents as well as aseptic techniques and ensure adequate growth environments. All liquids should be pre-warmed at least to room temperature. Pipette liquids gently along the sidewall into the well and avoid touching of the CCC after attachment until its translocation.

1. Reversible attachment of the CCC to the well bottom and cell seeding

Required material

- Sterilized forceps
- Pipettes
- PBS (pH 7.3 w/o Ca^{2+} / Mg^{2+})
- Tissue culture-treated multiwell plate suited for respective CCC diameter
- Cell culture medium

Intended use

The CCC is intended for research use only.

It is neither intended for human nor animal diagnostic, therapeutic use or any other clinical use.

Disclaimer

All data and recommendations correspond to the present state of our knowledge; they are published without engagement. We reserve the right to make alterations and additions in line with technical developments without prior notice. The customer is obliged to check whether our products meet his technical requirements.

Please contact us for questions or technical support:

Contact@bio.viscofan.com

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ATTACHMENT PROTOCOL

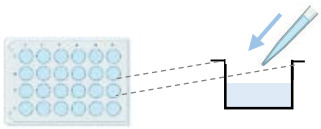
CCC diameter (well plate format)	Ø 34 mm (6-well)	Ø 21 mm (12-well)	Ø 14 mm (24-well)	Ø 10 mm (48-well)	Ø 7 mm (96-well)	Ø 88 mm (10 cm dish)
Volume of PBS	1000 µl	500 µl	250 µl	150 µl	50 µl	6 ml

STEP 1

Preload each well with PBS (pH 7.3 w/o Ca^{2+} / Mg^{2+}) in the volume given in the table above.

Important: Do not exceed these volumes!

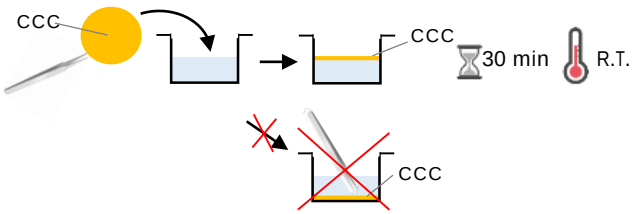
For cell types known to be sensitive to phosphate buffers, the CCC may be washed with ddH_2O in STEP 6 before incubation with medium.



STEP 2

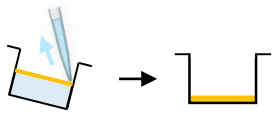
Remove the CCC carefully from the bag using a pair of sterile forceps and place it flat on top of the PBS. Incubate for 30 min at room temperature.

Important: Do not submerge the CCC!



STEP 3

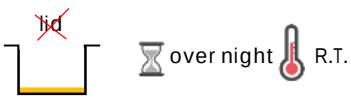
Carefully aspirate the PBS. Tip: tilt the well plate slightly to one side.
Then ensure that the CCC is positioned flat on the bottom of the well without wrinkles or entrapped bubbles.



STEP 4

Let the CCC dry overnight in the operating laminar flow hood with the lid of the well plate removed.

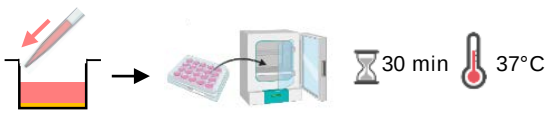
Important: thorough drying is necessary for reliable attachment of the CCC to the well bottom!



STEP 5

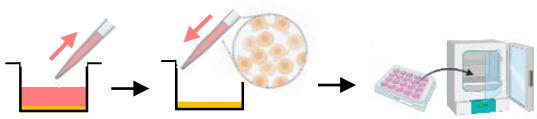
Prior to cell seeding, equilibrate the CCC with an appropriate volume of pre-warmed cell culture medium for at least 30 min at 37°C in the CO_2 incubator.

Important: Avoid touching the CCC until its translocation (if intended). Pipette liquids gently along the sidewall into the well.



STEP 6

Change the medium just prior to seeding cells on the CCC.



Most tool-symbols were derived from BioRender.

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2. Detachment of the cell-loaded CCC from the well bottom and transport

Required material

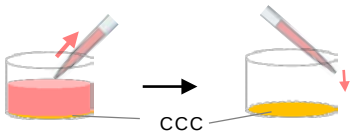
- (sterilized*) forceps
- pipette
- (sterilized*) glass slide or other support with smooth, wettable surface

**sterilization of tools is only needed if subsequent processes require sterile conditions.*

DETACHMENT PROTOCOL

STEP 1

Aspirate the medium from the well except for a small volume. Then wet the inner sidewall of the well with a small amount of medium.

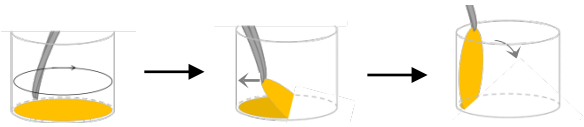


STEP 2

Optionally, circuit the CCC once at the well bottom with the tip of a pair of pointed forceps. Then grab the CCC at one side and pull it gently to the opposite well side in a flat angle. When the CCC is detached, lower it gently so that the cells are still facing the top. Then instantly proceed to step 3.

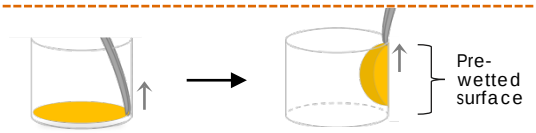
If the CCC does not come off easily, lift small areas of the CCC boundary at several sites before pulling.

Important: Proceed swiftly to Step 3 and use minimal medium to avoid membrane curling.



STEP 3

Pull the CCC along the wetted sidewall with the CCC outstretched flat against the sidewall and the adhered cells facing the center of the well.



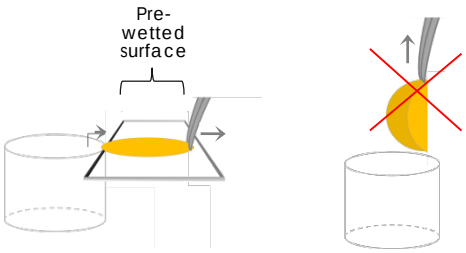
STEP 4

For transport we recommend to place a glass slide or a similar support close to the well, wetted with any buffer, water or medium. Carefully pull the CCC directly from the sidewall on top of the support.

Important: pre-wetting of the surfaces where the CCC is pulled along significantly facilitates gliding.

Avoid holding the CCC in the air with only one pair of forceps as this promotes interfolding!

Though re-unfolding is easily possible, folding or rolling up might disturb the adhered cells.



CCC-membranes for:	VWR Cat. No.
6-well plate	76518-082
12-well plate	76518-084
24-well plate	76518-086
48-well plate	76518-088
96-well plate	76518-090

Contact us
contact@bio.viscofan.com
+49 (06201) 86-358

Viscofan BioEngineering
A Business Unit of Naturin Viscofan GmbH
Badeniastraße 13 • 69469 Weinheim, Germany
Phone +49 (0)6201 86-358 • Fax +49 (0)6201 86-226
contact@bio.viscofan.com • www.viscofan-bioengineering.com

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