

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 below 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.

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. Pipet liquids gently along the sidewall into the well and avoid touching of the CCC after attachment until its translocation.



Collagen Cell Carrier for cell/tissue transfer

Please note: as an alternative product format, CCCs (RTU) “Ready-to-use” can be purchased already attached to a multiwell plate. However, as the CCCs (RTU) “Ready-to-use” tend to stick more firmly, we recommend to use single-packed CCCs, if translocation after cell seeding is intended.

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

Materials needed:

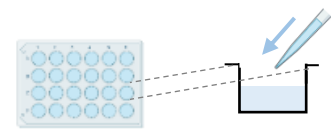
- Sterilized forceps
- Pipettes
- PBS (pH 7.3 w/o Ca²⁺ / Mg²⁺)
- Tissue culture-treated multiwell plate suited for respective CCC diameter
- Cell culture medium

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 Ca²⁺ / Mg²⁺) in the volume given in the table above.

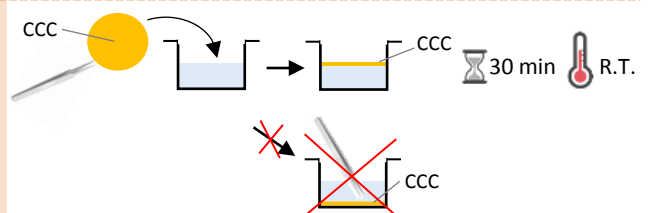
Important: Do not exceed these volumes. For cell types known to be sensitive to phosphate buffers, the CCC may be washed with ddH₂O in STEP 6 before incubation with medium.



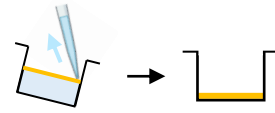
STEP 2

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

Important: Do not submerge the CCC



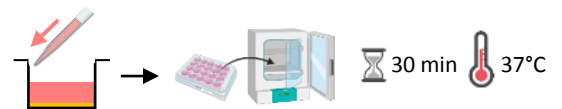
STEP 3 Carefully aspirate the remaining PBS. Tip: tilt the wellplate slightly to one side. Then ensure that the CCC is positioned flatly on the bottom of the well without wrinkles or entrapped bubbles.



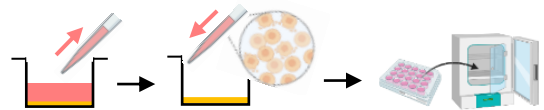
STEP 4 Let the CCC dry overnight under the operating laminar flow hood with the lid of the well plate removed. **Important: thorough drying is necessary for reliable attachment of the CCC to the well bottom.**



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 CO₂ incubator. **Important: Avoid touching the CCC until its translocation (if intended). Pipet liquids gently along the sidewall into the well.**



STEP 6 Change the medium just prior to seeding cells on the CCC.

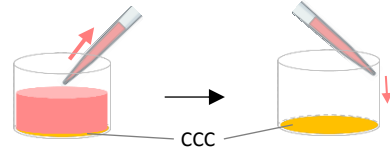


Detachment of the cell-loaded CCC from the well bottom and transport

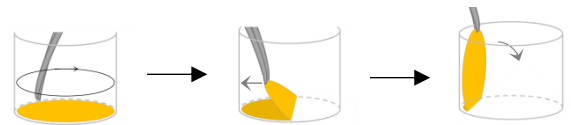
Materials needed:

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

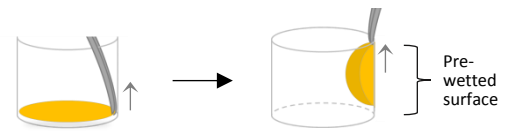
STEP 1 Aspirate the medium from the well except for a small volume. Then wet the inner sidewall of the well with a small amount of medium.



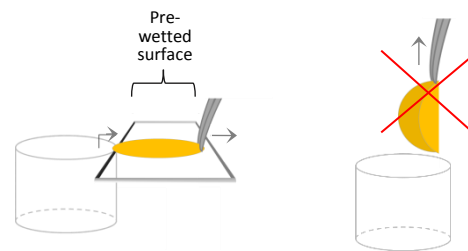
STEP 2 Optionally, circuit the CCC once at the well bottom with the tip of a pair of pointed forceps. Then grab the CCC at one side and remove it from the bottom of the well by pulling gently to the opposite well side in a flat angle. If the CCC doesn't come off easily, lift small areas of the CCC boundary at several sites before pulling.



STEP 3 When the CCC is detached, instantly pull it along the wetted sidewall with the CCC outstretched flatly 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 helps easy gliding of the CCC. Avoid holding the CCC in the air with only one pair of forceps as this promotes interfolding.** Though re-unfolding is easily possible, folding might disturb the cells adhered.



Most tool-symbols were derived from BioRender.

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