

Collagen Cell Carrier (CCC)

Before starting please note

When working with CCC 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. Add liquids gently along the sidewall into the well.

Before cell seeding, please follow the protocol below to (reversibly) attach the CCC to the well bottom. After attachment avoid touching of the CCC membrane until its translocation (if intended).



Later during cell culture, the cell-seeded CCC may be removed from the well and transferred to various subsequent processes (e.g. implantation, embedding for histological analyses etc.). For this, please follow the CCC detachment protocol at the end.

Collagen Cell Carrier (CCC) in R&D quality is intended for research use only. It is neither intended for human nor animal diagnostic, therapeutic use or any other clinical uses!

Corresponding documents:

- Product Data Sheet - Collagen Cell Carrier (CCC)
- Application Note - Detachment of cells cultured on fibrous collagen surfaces
- Application Note - Staining of cells grown on fibrous collagen surfaces with the life cell tracking dye BDTM DiIC12(3)

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 with his own technical requirements. We shall be glad to answer any queries.

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Attachment of the CCC to the well bottom and cell seeding

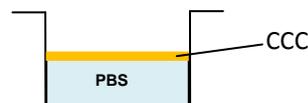
Materials needed:

- Sterilized forceps
- pipettes
- PBS (pH 7.3 w/o Ca^{2+} / Mg^{2+})
- Multiwell plate, suited for adherent cells in respective CCC format
- Cell culture medium

Well plate	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 dishes)
Volume of PBS	1000 µL	500 µL	250 µL	150 µL	50 µL	6 mL

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 rehydrated with ddH₂O in step 6 before incubation with medium.
2. Take the CCC out of the blister by using a pair of sterile forceps and place it on top of the liquid.

Important: Do not submerge the CCC.



3. Incubate for 30 min at room temperature.
4. Carefully aspirate the remaining PBS.
Assure that the CCC is positioned flatly on the bottom of the well without wrinkles or entrapped bubbles.
5. Let the CCC dry overnight under the operating laminar flow hood with the lid of the well plate open.
Important: thorough drying is necessary for reliable attachment of the CCC to the well bottom.
6. Prior to cell seeding, equilibrate the CCC with an appropriate volume of pre-warmed medium for at least 30 min at 37°C in the CO₂ incubator.
7. Change the medium just prior to seeding cells on the CCC.

Detachment of cell-loaded CCC from the well plate

Materials needed:

- (sterilized) forceps
- pipette
- (sterilized) glass slide or other support

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

User Protocol

2. Optionally, circuit the CCC once at the well bottom with the tip of a pair of pointed forceps (a). Grab the cell-scaffold complex at one side and remove it from the bottom of the well by pulling gently to the opposite well side in a flat angle (b, c). If the CCC doesn't come off easily, remove small areas of the CCC boundary at several sides before pulling.
3. When the CCC is detached, instantly pull it along the sidewall with the CCC outstretched flatly against the sidewall and the cells facing the inside of the well (e).
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 (f).

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.

