

Collagen Insert

Product Description

Collagen Inserts contain a self-supporting non porous membrane of pure, non-cross-linked bovine collagen type I for cell cultivation. The collagen membrane can be easily separated from the insert structure by removing the clamping ring.



Applications

The Collagen Insert contains a robust, sterile collagen membrane for the growth and differentiation of various cell types, representing an *in-vivo* like collagen for use in conventional 6-well plates. It is highly 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 detachment procedures can be used to detach adherent cells from the Collagen Insert.

Immunofluorescence

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

The staining procedure can be carried out directly on the cell covered scaffold, and can be followed directly by embedding.

Histological analysis

Fixation of cells on the Insert membrane can be performed by standard fixation protocols using paraformaldehyde, buffered formaldehyde, acetone, glutaraldehyde, or methanol.

The Insert membrane can be frozen or embedded in paraffin or epoxy resins (e.g. EPON) and sliced with a cryostat or microtome, respectively. The scaffold is also suitable for electron microscopic investigations.

Implantation

The collagen membrane exhibits 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 viability and growth on the insert membrane can be monitored by colorimetric methods (tetrazolium salt based dyes e.g. WST-1, MTT test) according to the manufacturer's recommendations.

Storage

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

Shelf life: 24 months

**PLEASE NOTE: COLLAGEN INSERTS ARE INTENDED FOR IN VITRO RESEARCH USE ONLY!
NOT FOR USE IN DIAGNOSTIC, THERAPEUTIC OR CLINICAL PROCEDURES.**

BEFORE STARTING, PLEASE NOTE:

When working with Collagen Insert, 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.

Always add liquids into the Insert along the sidewall.

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Cell seeding in the Collagen Insert

PLEASE NOTE: Carry out all steps in a sterile environment!

1. Unpack the provided 6-well plate and open a blister unit containing a sterile Collagen Insert. Remove the insert from the blister using a pair of sterile forceps. Place the Insert in a well.
2. Wash the collagen membrane inside and outside by adding pre-warmed PBS in the Insert and underneath. Incubate for 30 minutes at room temperature.
For the removal of buffer underneath the Insert a Pasteur pipette is best suited. Avoid piercing of the membrane!
3. Prior to cell seeding, the collagen of the Insert needs to be equilibrated with the appropriate volume of pre-warmed medium for at least 10 min at 37°C (inside the Insert and underneath).
For cell types known to be sensitive to phosphate-buffers, the Insert collagen membrane may be washed with ddH₂O before incubation with medium.
4. Aspirate the medium just prior to seeding cells into the Collagen Insert.
5. It is important to avoid bulging of the membrane either upwards or downwards to ensure uniform cell growth. This can be achieved by adding ~2,4 mL of cell-containing medium into the Insert and ~4 mL of medium underneath. If required, the bulging of the membrane can be monitored easily in a phase contrast microscope and may be optimized by adding medium to the inside and/or outside of the Insert.