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Product Information

HyStem™-C 96 Well Plate

Catalog Number **H2666**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

The HyStem™-C 96 Well Plate provides the HyStem-C formula as a convenient, lyophilized "sponge" pre-filled in a 96 well plate. The sponge retains its porous structure when rehydrated.

The plate is ideal for culturing primary cells and cell lines in 3D or tissue engineering applications requiring a defined pore structure for efficient gas exchange and fluid transfer.[1-3] It provides a basic scaffold for 3D cell growth that closely matches the extracellular environment. It is suitable for a broad range of cell culture applications, including the cultivation of hepatocytes and primary human tracheal scar fibroblasts.[4]

Unlike animal-derived extracellular matrices (ECM), HyStem-C sponge is based on three biocompatible, chemically defined components:

HyStem – a thiol-modified hyaluronan (a major constituent of native ECM), carboxymethyl hyaluronic acid-thiopropanoyl hydrazide (CMHA-S, CMHA-DTPH, carboxymethyl hyaluronic acid-DTPH)

Gelin-S™ – a thiol-modified gelatin (denatured collagen), carboxymethyl gelatin-thiopropanoyl hydrazide (GTN-DTPH, carboxymethyl gelatin-DTPH)

Extralink™ – a thiol-reactive crosslinker, polyethylene glycol diacrylate (M_W = 3,400 g/mole, PEGDA)

The lyophilized formula contains 33 wt% HyStem, 33 wt% Gelin-S, and 33 wt% Extralink, and is ready-to-use with a pre-filled volume of 100 µl in each well.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store the HyStem-C 96 Well Plate at –20 °C for up to one year.

Do not break the sealed packaging since this protects the product from moisture. Breaking the packaging will decrease the shelf life of the product.

Procedure

1. Prepare cells for use as per standard procedures. Seeding density varies with cell type. Experimentation may be required to determine the optimal seeding density for the culture of interest.

2. Prepare the 96 well plate by removing it from its packaging in aseptic conditions.

3. Pipette 100 µl of cells and medium at the appropriate seeding density on top of the sponge. While pipetting, distribute the cells across the entire area of the sponge surface. If necessary, add more medium to ensure the entire sponge is thoroughly wetted. The sponge should absorb the cells and medium and there should be no excess liquid visible in the bottom of the well.

4. Place the plate in a 37 °C incubator with 5% CO_2 for 0.5–1 hour to allow the cells to attach.

5. After 1 hour, add the appropriate volume of medium to the well. Place in a 37 °C incubator with 5% CO_2.

6. Change the medium as required using the following steps:
   - Carefully aspirate off the medium from above the sponge.
     Note: The sponge can be removed by the vacuum as well, so this must be done carefully.
   - Add the appropriate volume of fresh medium to each well without disrupting the sponge. Return the plate to a 37 °C incubator with 5% CO_2.

References

1. Liu, Y. et al., Disulfide-crosslinked Hyaluronan-Gelatin Sponge: Growth of Fibrous Tissue In Vivo, J. Biomed. Mat. Res., **68A**, 142-149 (2004).
2. Liu, Y. et al., Accelerated repair of cortical bone defects using a synthetic extracellular matrix to deliver human demineralized bone matrix. J. Orthop. Res., **24**(7), 1454-1462 (2006).
3. Liu, Y. et al., Osteochondral Defect Repair with Autologous Bone Marrow-Derived Mesenchymal Stem Cells in an Injectable, *in situ* Crosslinked Synthetic Extracellular Matrix. Tissue Eng., **12**, 3405-3416 (2006).
4. Serban, M.A. et al., Effects of Synthetic Extracellular Matrices on Primary Human Fibroblast Behavior. Acta Biomaterialia, **4**, 67-75 (2008).

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