Sigma-Aldrich®

Data Sheet

TrueGel3D™ HTS Hydrogel Plate

3D Cell Cultureware

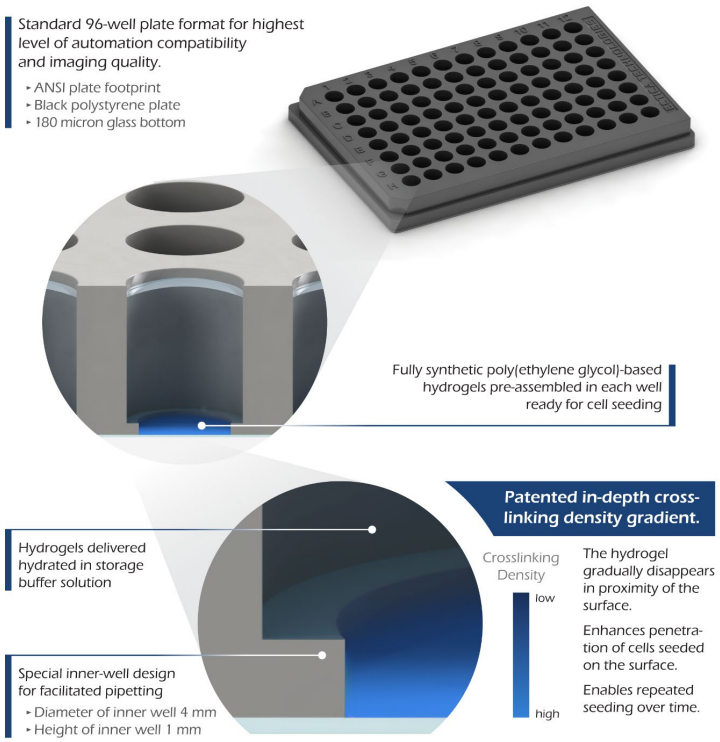
Cat. # TRUE-HTS1 **pack size: 1 Plate**

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES
NOT FOR HUMAN OR ANIMAL CONSUMPTION

Store at: Room Temperature

Background

The TrueGel3D™ HTS Hydrogel Plate is a ready-to-use solution to easily establish 3D cell cultures using fully synthetic hydrogels in a simple and automation-compatible manner. The 96-well polystyrene glass bottom plates contain precasted synthetic functionalized PEG based hydrogels. These innovative hydrogels contain gradually increasing crosslinking densities throughout the well. Users can proceed directly with seeding cells without any hydrogel preparation or encapsulation steps. After cell seeding, cells will gradually infiltrate the hydrogel and establish a 3D cell culture environment within days. Furthermore, this newly engineered hydrogel surface gives the user the possibility to sequentially establish co-culture systems seeding different cell populations at different time-points in the same hydrogel.



References

Benjamin R Simona. Soft Hydrogels Featuring In-Depth Surface Density Gradients for the Simple Establishment of 3D Tissue Models for Screening Applications. SLAS Discov. 2017 Jun;22(5):635-644.

Storage and Handling

Store all components of the TrueGel3D™ HTS Hydrogel Plate at room temperature (do not freeze) away from direct sources of light and heat. Do not store upside down.

Quality Control Testing

Plate Cleanliness: Particle count/well by microscope inspection <10
Hydrogel Transparency: Pass
Hydrogel Thickness: Pass
Microbiological Analysis: Negative for Microorganisms according to ISO 11737-1

The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the US and Canada.

EMD Millipore Corporation, 28820 Single Oak Drive, Temecula, CA 92590, USA 1-800-437-7500

Ver 1.0/2020-10-14/TRUE-HTS1-DS/NJA

Protocols

General Protocol for Cell Seeding and Culture Maintenance

Note: Before starting please verify the good status of the plate. The polyethylene (PE) pouch must be intact with no visible liquid inside. The plate must be intact inside the pouch. Open the PE pouch with scissors or a scalpel under the sterile laminar flow bench. Once removed from the pouch, the plate is still sealed on its top-side with a polypropylene (PP) adhesive foil which is keeping the content inside each of the 96 wells. From the glass bottom side, verify that the glass cover is intact and that the gels look transparent, like if the wells were filled with water.

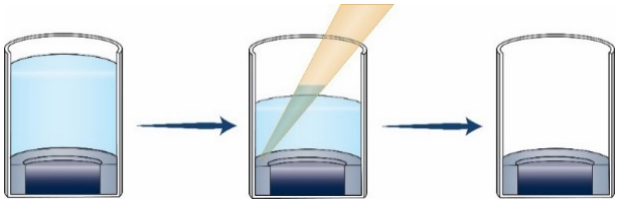
1. **Warm assay plate**. Make sure the plate was at room temperature for at least 30 minutes prior use.

2. **Prepare the cells and media to be used.**

3. **Remove the sealing adhesive foil.** Carefully peel off the sealing tape from the assay plate. We suggest to firmly hold the plate with the left hand on the table and peel off the sealing foil with the right hand starting from the top-right angle of the plate and slowly moving to the bottom-left angle of the plate. A liquid meniscus may form on top of the wells due to the negative pressure applied by removing the foil, but this meniscus pops and disappears within seconds.

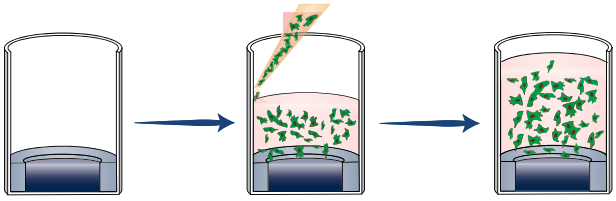
4**. Aspirate the storage buffer.** Insert the pipet tip in the well and descend along the side wall. Place the pipet tip on the plastic ring inside the well and carefully aspirate the storage saline buffer. It is normal to perceive a small suction resistance. Slightly lift the pipette tip and the liquid will be aspirated.

Note: Do not touch or aspirate right over the gel because of the risk of damaging it. It is possible to use multichannel pipets or pumps (low suction pressure) to accelerate the process. It is acceptable If some storage buffer remains on the well because it is a Tris buffer which won't negatively affect you culture development. Minimize time between step 4. and 5. to reduce the risk of drying the gel.

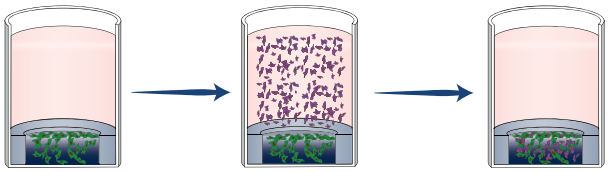


5. **Add cells and medium.** Add the cell suspension with a maximum volume of 200µL in each of the wells. Transfer the plate to the incubator. Keep cells in culture and change culture medium every 2nd day applying similar pipetting techniques as in step 4 and 5 to establish a 3D cell culture environment.

Note: Residual trace activity of trypsin can result in digestion of the hydrogel. Especially when using serum free media, after detaching the cells, make sure to inhibit trypsin using a trypsin inhibitors or inactivating solutions (i.e. soybean). Optimal cell seeding density depends on culture types and readouts. To better control the concentration of your medium components, it is possible to soak and rinse the hydrogel with the culture medium before the addition of the cells.



6. **Sequential seeding of cells (optional)**. At a desired time point, remove medium and add a second cell population in co-culture medium (maximum volume of 200µL per well). Keep in culture until assay end. Change medium every other day.



Cell Retrieval for Passaging and Analysis

1. Digest gels with 60 µL/well collagenase A (1 mg/mL,) for 30 min at 37°C.
2. Collect cells in 1.5 mL Eppendorf tubes.
3. Retrieved cells can be used for FACS, RNA/DNA extraction or further assays.

Note: Other collagenases or enzyme cocktails can be used to retrieve the cells (i.e. liberase or trypsin).

Fixation and Cell Staining

1. Remove the 200uL of cell culture medium and fix with 200 µL/well of 4% paraformaldehyde in PBS.
2. If antigen blocking is required add 200uL/well of blocking buffer composed of 5% Donkey Serum and 0.3 % Triton X100 in PBS (if cell permeabilization is required) and incubate for 2h at RT.
3. Add 200uL/well of PBS and wait 5 min at RT without shaking. Discard the PBS and repeat 3X.
4. Incubate over night at 4°C with 100-150uL/well of primary antibody solution in blocking buffer.
5. Repeat step 2.
6. Incubate 2h at RT with 100-150uL/well with secondary antibody solution in blocking buffer.
7. Repeat step 2.

Note: For extracellular matrix (ECM) protein staining, it is recommended to add the primary antibody to the cell culture medium prior to fixation, at least 3 hours in the incubator. Afterwards, follow the guidelines for fixation and staining.

Representative Data

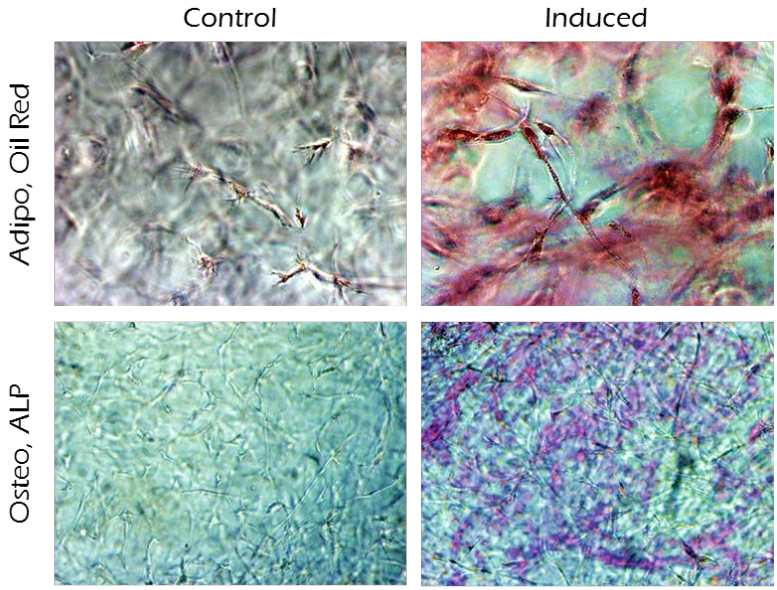


Figure 1. Mesenchymal Stem Cell Differentiation. 15,000 bone-marrow derived mesenchymal stem cells/well were seeded in the TrueGel3D-HTS Hydrogel Plate in 200 µL of invasion medium for 4 days (MEM-alpha containing 10% FBS, 1% Pen/Strep and 10 ng/mL PDGF). Followed by 15 days in osteogenic or adipogenic differentiation media. Cell differentiation was analyzed using Oil Red O, Sigmafast ALP substrate, or immuno-staining for lipid droplets (Bodipy) or Alkaline phosphatase antibody.

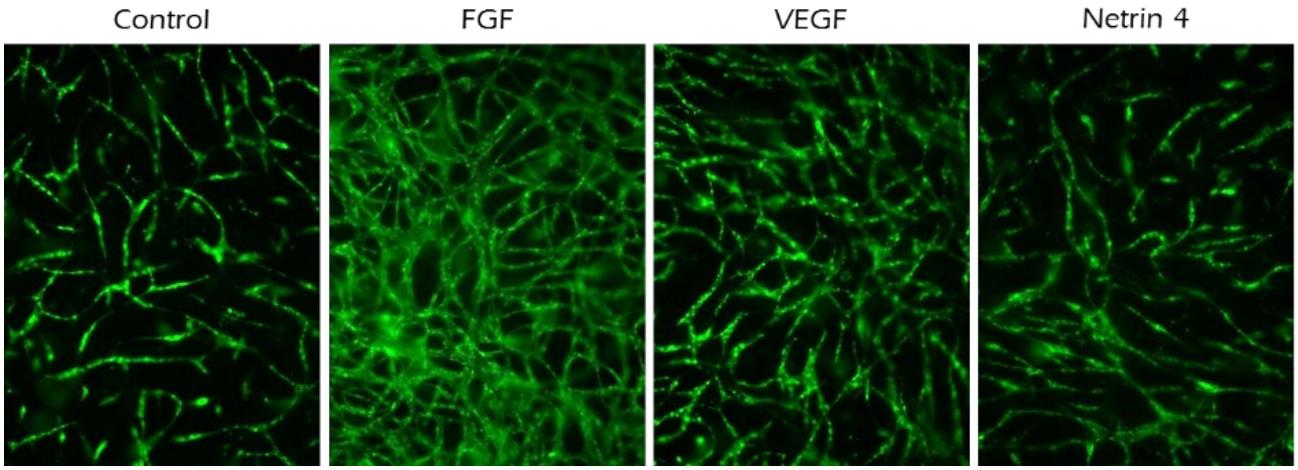


Figure 2. Angiogenesis Assays. 10,000 GFP labeled endothelial cells (HUVECs) were seeded in the TrueGel3D-HTS Hydrogel Plate in 200 µL of growth medium (70% MEM-alpha, 20% EGM-2, 10% FBS, 1% Pen/Strep and 10 ng/mL FGF). FGF, VEGF or Netrin-4 was added to media on the following day to evaluate their angiogenic potential. Angiogeneis was analyzed at day 10 by imaging and quantitfying endothelial cells tubule length.

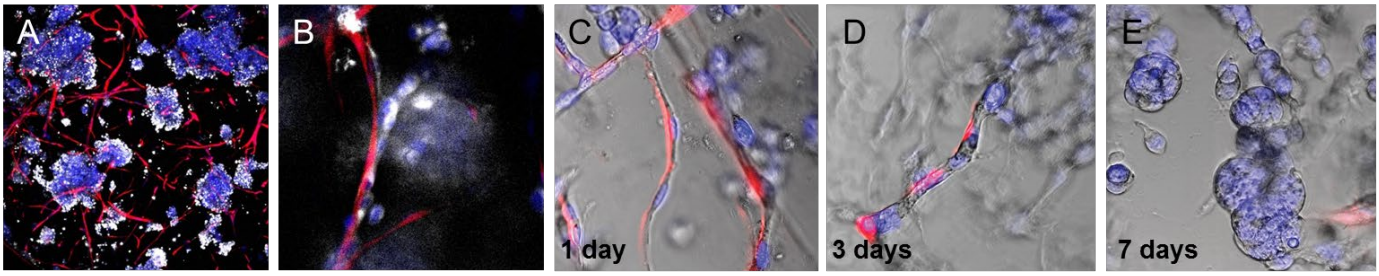


Figure 3. Tumor Cell Microenvironment. 10,000 RFP labeled fibroblasts were seeded in the TrueGel3D-HTS Hydrogel Plate in 200 µL of invasion medium (MEM-alpha containing 10% FBS, 1% Pen/Strep and 10 ng/mL PDGF). After 5 days of pre-culture, 7,000 pancreatic carcinoma cells (MIA-Paca) were seeded to create co-culture and maintained for up to 2 weeks. Top view at hydrogel surface (A) and close-up inside the gel (B). Bright-field showing growth and invasion of MIA-Paca cells in association with pre-seeded fibroblasts during the first week of cancer cell culture (C-E).

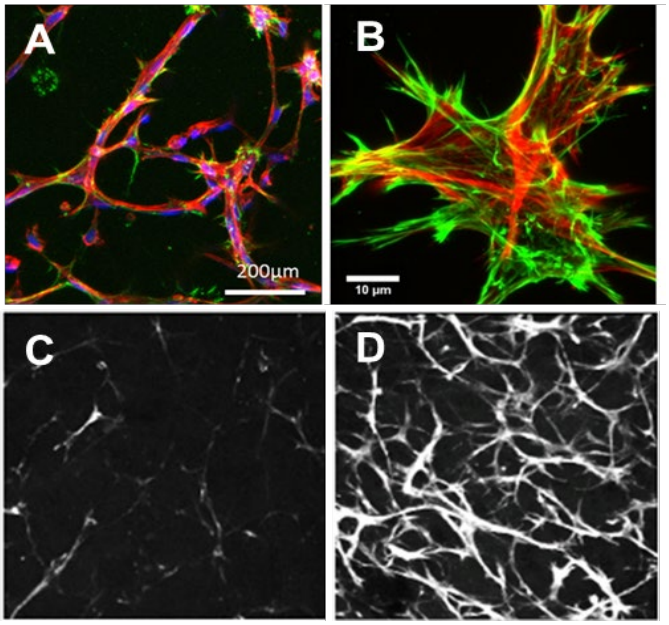


Figure 4. Extracellular Matrix Deposition. 10,000 RFP labeled fibroblasts were seeded in the TrueGel3D-HTS Hydrogel Plate in 200 µL of invasion medium (MEM-alpha containing 10% FBS, 1% Pen/Strep and 10 ng/mL PDGF). The following day compounds to steer the ECM were added to the culture media. Change medium every 3rd day. (A, B) Confocal micrographs of fibroblasts (red) and endogenous fibronectin (green). (C, D) Confocal micrographs of collagen deposited by fibroblast culture for 14 days without (C) or with (D) 50 g/L ascorbic acid.