Extracellular Matrix Data Sheets

PRODUCT NAME

Extragel Matrix , Phenol Red-free, Standard

PRODUCT NUMBER

AMS.SEM-S01

PRODUCT INTRODUCTION

The basement membrane is a matrix under the basal surface of epithelial cells of animals. Extragel is a reconstituted matrix hydrogel formed by basement membrane components extracted from mouse tumor tissues. This matrix hydrogel is mainly composed of laminin, collagen IV,and heparan sulfate proteoglycans (Kleinman et al. 1986). Besides, it contains various growth factors, such as epidermal growth factor (EGF), platelet-derived growth factor (PDGF), nerve growth factor (NGF), basic fibroblast growth factor (FGF-2), transforming growth factor-β (TGF-β), and insulin-like growth factor (IGF) (Vu-kicevic et al. 1992).

PRODUCT CHARACTERISTICS

Extragel is liquid at 4°C but gelled when heated to 37°C. This transformation phenomenon is reversible. It can be liquefied again when it is stored at 4°C overnight. (**Tip:** It is recommended to store the Extragel in an ice box in a refrigerator at 4°C to realize the full liquefaction of the reconstitute matrix hydrogel.)

STORAGE CONDITION

Dispense Extragel into appropriate aliquots. Stable for 2 years when stored at -80°C. -20°C freezer storage is ideal for short-term storage.

PRODUCT APPLICATION

This product can be applied to the growth, differentiation, metabolism and toxicology of organoids, in vivo and in vitro angiogenesis experiments.

PRECAUTIONS

Extragel would start solidifying after the temperature is higher than 10°C, so the operation should be performed on ice. The matrix hydrogel can be dissolved in basic culture medium pre-chilled at 4°C, and the organoid can be released from the Extragel.

SPECIFICATIONS

Concentration	8-12 mg/mL
Product type	Basement Membrane Matrix
Sterility	Sterile
Endotoxin Level	<2 EU/mL
Quality Grade	Cell Culture Grade
Shipping Condition	Dry Ice
Product Line	AMSBIO
Form	Frozen
Shelf Life	24 months
Quantity	1 mL

Format	Tube(s)
Cell Type	Primary epithelial cells, endothelial cells, human embryonic stem cells (hESC), human induced pluripotent stem cells (iPSC)
Serum Level	Serum Free
LDEV PCR Test	LDEV Free
Mycoplasma PCR Test	Mycoplasma Free

OPERATION METHOD

Tumor organoid drug sensitivity experiment (2 hours)

1. Suspend the tumor organoids (experimental group) and normal organoids (control group) in pre-cooled basic culture medium at 4°C. Slowly mix to promote gel liquefaction, and maintain the structure of the organ intact for suspension culture in a 5% matrix gel solution (Guillen et al. 2022).
2. Collect organoid cells by centrifugation and perform cell counting.
3. Add Extragel into tube and mix with cells.
4. Add the mixture of cells and Extragel to a 96-well plate or a 384-well plate via pipetting and immediately put the plate into an incubator.
5. After about 10 minutes, the Extragel will solidify, and add the culture medium to the well with matrix and cells.
6. After the formation of organoids (if it is mechanical blowing, organoids will form after 24 hours; if it is enzyme digestion, organoids will form in 3-5 days), add PI dye and different types and concentrations of anti-tumor drugs to each well for drug sensitivity evaluation.
7. Use high-content microscopy for live cell imaging to determine the sensitivity of tumor organoids to various drugs.

Angiogenesis experiment (using immortalized HUVEC cell line as an example, 1 hour)

1. Replace complete medium with starvation cell culture medium, the starvation cell culture medium contains DMEM medium containing 0.2% FBS, 2 mM L-glutamine, 1 mM sodium pyruvate, 100 U/mL penicillin, and 100 μg/mL streptomycin to culture cells for 24 hours.
2. Evenly spread 50 μL Extragel on the bottom of a 96-well plate. Note: the pipette tip should be pre-cooled for half an hour. Operate on ice as much as possible to avoid premature solidification of the Extragel and the formation of bubbles.
3. Incubate the 96-well plate in incubator for 30 minutes to solidify the Extragel.
4. Digest HUVEC cells and count them.
5. Add 200 μL of HUVEC cell suspension (containing 5X10^4 cells) to the 96-well plate containing the matrix gel. Put the 96-well plate in the incubator.
6. The vascular-like network structure will form in 3 to 12 hours.
7. Carefully remove the culture medium at the optimal time of vascular network formation, stain with culture medium containing live cell dye 1/1000 Calcein AM (green), and take pictures with a microscope.

Neurite 3D outgrowth experiment (using rat embryonic neural stem cells as an example, 2 hours)

1. Take rat embryos at 12-15 days of gestation, and use scissors and forceps to separate the cerebral cortex into pre-cooled DMEM culture medium at 4°C.
2. Use a 70μm filter to obtain a single-cell suspension, and perform cell counting.
3. Centrifuge (300 g, 3 min), discard the supernatant. Mix the cells with Extragel, and then add the gel mixture to a 24-well plate, 50μl per well.
4. Put the 24-well plate in the incubator. After about 10 minutes, the Extragel will solidify. Add 1 mL of

neural differentiation medium: Neurobasal medium, 2% B27, 2 mM L-glutamine, 5% FBS, 20 ng/mL EGF and 20 ng/mL bFGF, 100 U/mL penicillin, and 100 μg/mL streptomycin. From the 2nd day, obvious neurite outgrowth is observable.

5. A large number of neurites can be observed on the 7th day.

APPLICATION CASES

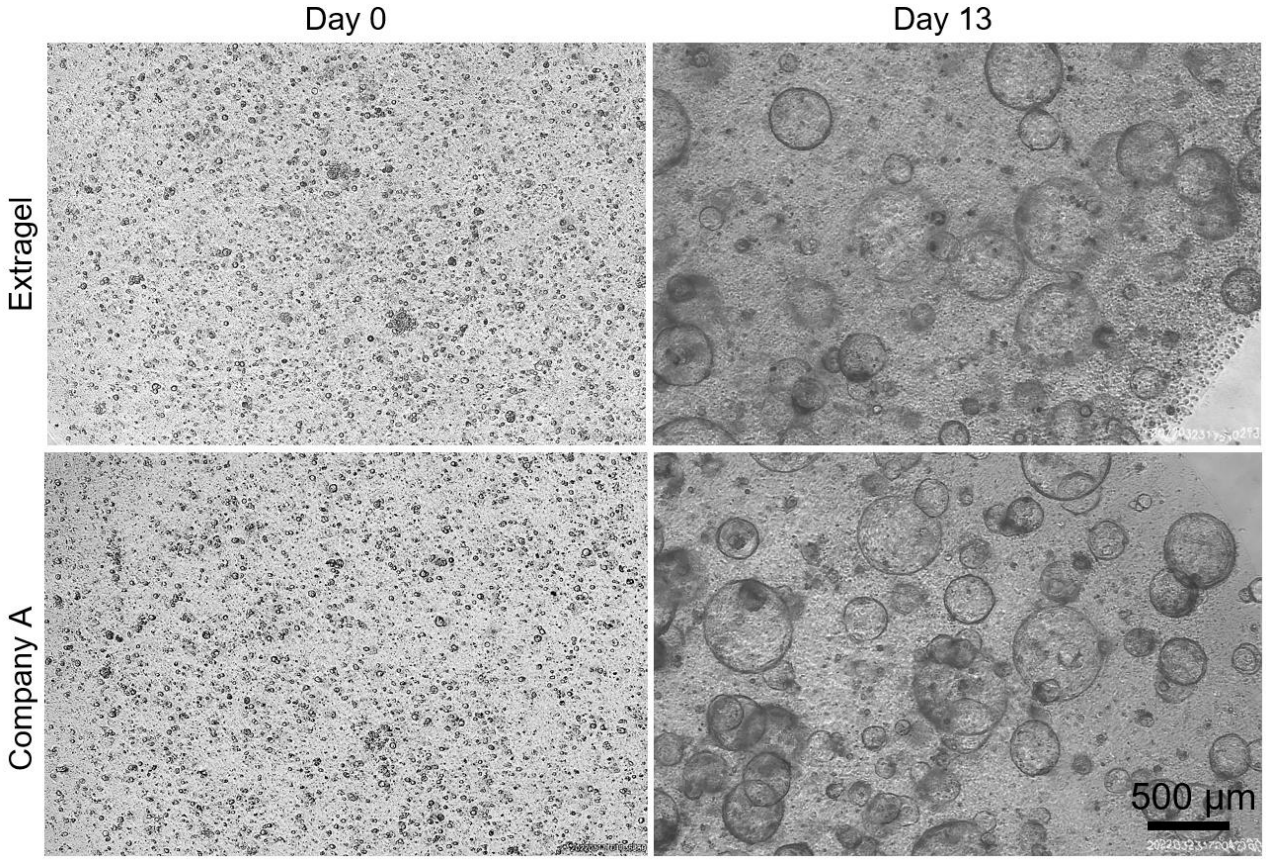


Figure 1. Growth of human primary colon cancer organoids in the matrix gel of Company A and Extragel.

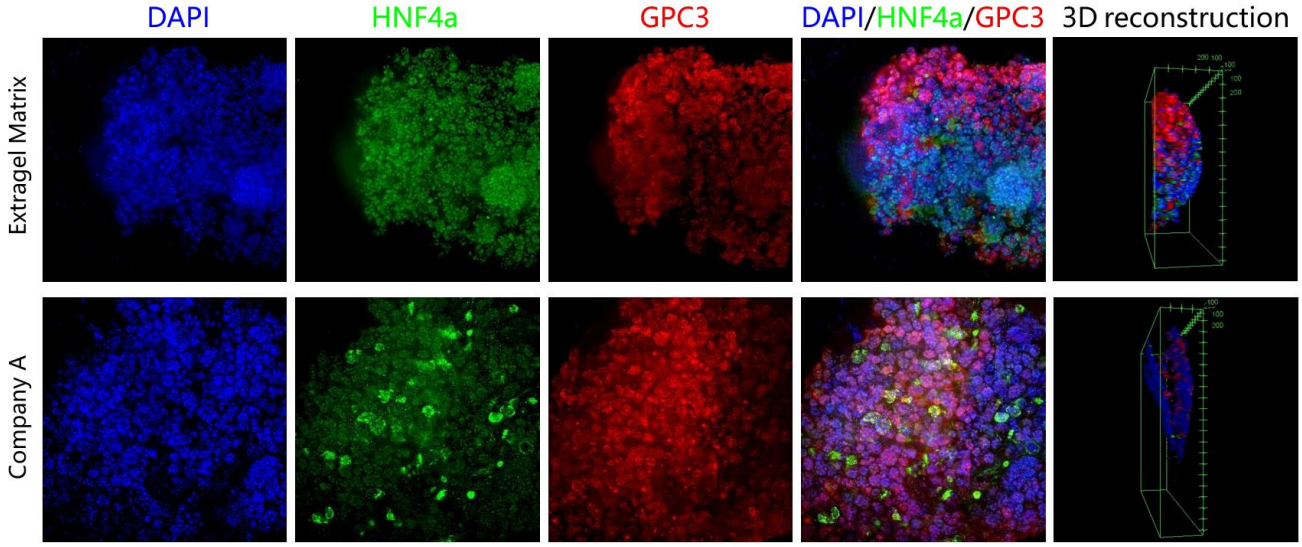


Figure 2. Growth of hepatocellular carcinoma organoids in the matrix gel of Company A and Extragel. Hepatocellular carcinoma organoids were stained with nuclear dye DAPI (blue) and antibodies against liver cell biomarker HNF4a and hepatocellular carcinoma marker GPC3.

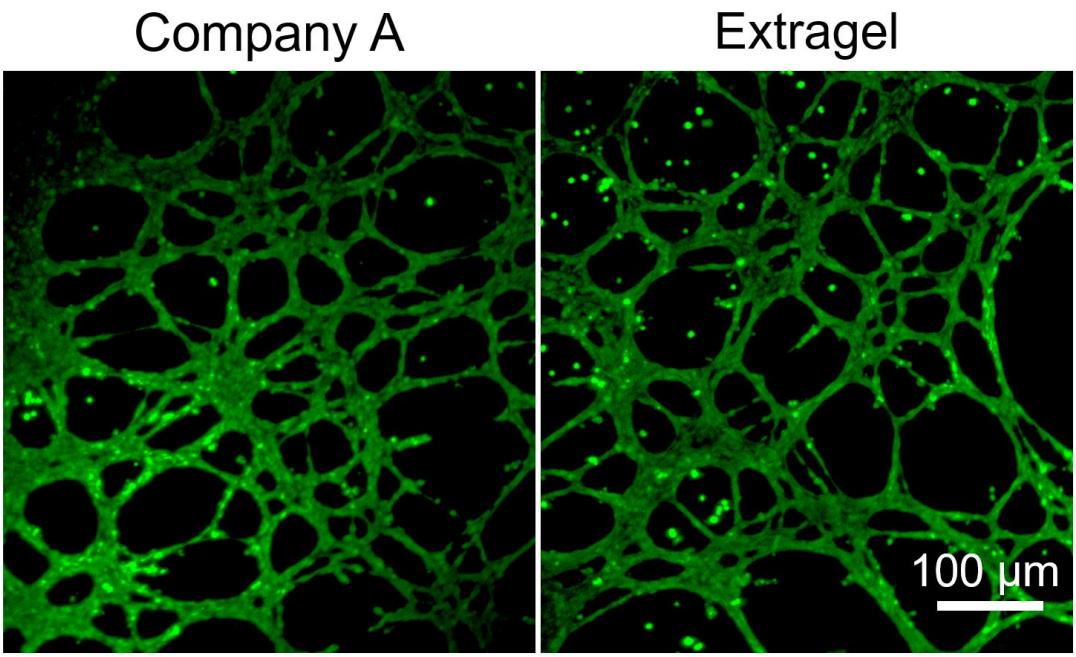
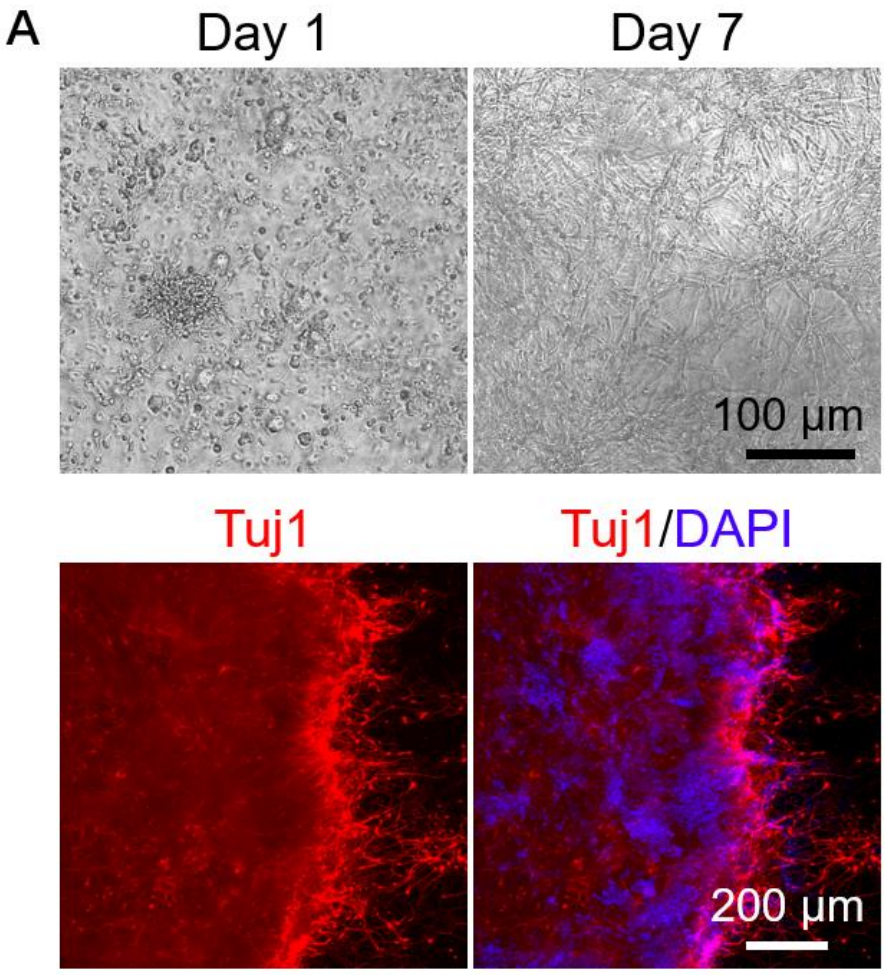


Figure 3. Human umbilical vein endothelial cells (HUVEC) can form vascular networks on the matrix gel of Company A and Extragel. HUVEC cells were stained with live cell dye Calcein AM (green) for imaging.



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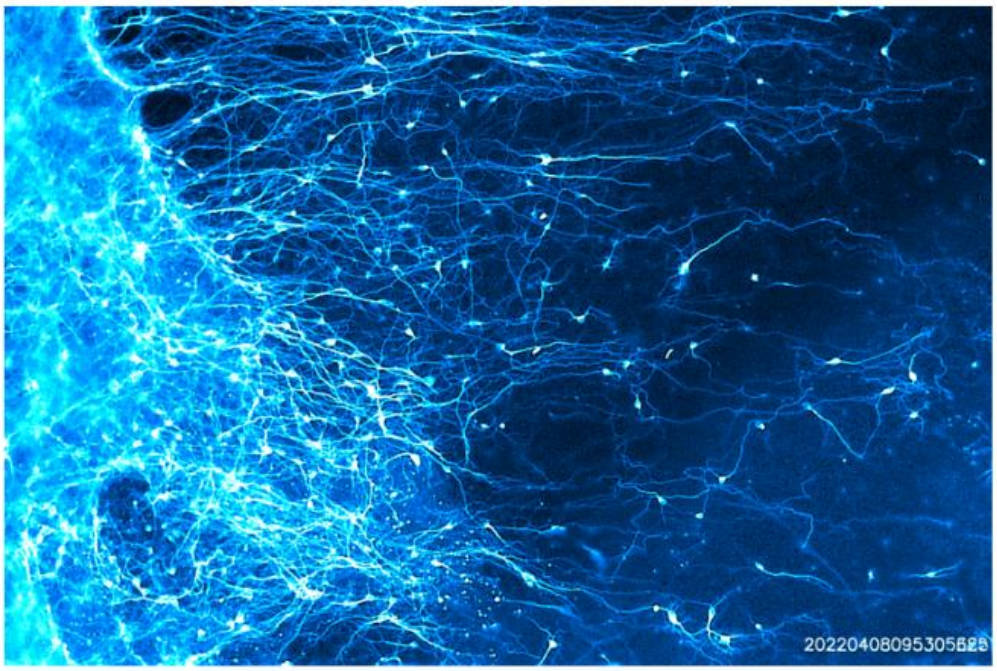

Figure 4. Rat neural stem cells can grow and differentiate in Extragel, forming a neurite network. Rat neural stem cells were cultured in Extragel for 7 days and were stained with antibodies against neural cell marker Tuj1 (red) and nuclear dye DAPI (blue) for imaging (A). The bottom image shows a pseudo-color photo of neurons (B). This experiment can be used for neurotoxicity and neuronal differentiation and development evaluation.

REFERENCE

1. Kleinman HK, et al, Basement membrane complexes with biological activity. Biochemistry 25: 312 (1986).
2. Vukicevic, Slobodan, et al. Identification of multiple active growth factors in basement membrane Matrigel suggests caution in interpretation of cellular activity related to extracellular matrix components. Experimental cell research 202: 1 (1992).
3. Guillen, K P, et al. A human breast cancer-derived xenograft and organoid platform for drug discovery and precision oncology. Nature Cancer 3: 232 (2022).