

Extragen Data Sheet

PRODUCT NAME:

Extracellular Matrix Hydrogel (Extragen), Phenol Red-free, Reduced Growth Factor

PRODUCT NUMBER

AMS.SEM-I01

PRODUCT INTRODUCTION

The basement membrane is a matrix under the basal surface of the epithelial cells of animals. Extragen 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

Extragen 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 Extragen in an ice box in a refrigerator at 4°C to realize the full liquefaction of the reconstituted matrix hydrogel.)

STORAGE CONDITION

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

PRODUCT APPLICATION

This product is suitable for expanding and maintaining human embryonic stem cells (hESC) and induced pluripotent stem cells (iPSC) using a feeder-free method.

PRECAUTIONS

Extragen 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 a basic culture medium pre-chilled at 4°C, and the organoid can be released from the Extragen.

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 |
| Form | Frozen |
| Shelf Life | 24 months |

| | |
|---------------------|--|
| Quantity | 1 mL |
| Format | Tube(s) |
| Cell Type | 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

Organoid culture (1 hour)

1. Thaw the Extragel (SEM-R01) in a refrigerator at 4°C overnight.
2. Preheat the 24-well plate in the cell culture incubator.
3. Prepare aliquots of Extragel using pre-chilled tips.
4. Prepare the single-cell pellet with 1×10^5 cells derived from patients or animal tissue, centrifuged at 300 g for 5 minutes.
5. Mix the cell pellet with 50 μ L Extragel thoroughly.
6. Add the mixture into the well of a plate (50 μ L per well).
7. Keep the plate in the incubator for 10 minutes, flip, and keep after another 5 minutes.
8. Add 500 μ L culture medium to the well with matrix and cells.
9. Change the medium every 3 days.

Feeder-free culture method for human embryonic stem cells (hES) and induced pluripotent stem cells (iPSC) (1 hour)

1. Thaw the matrix gel in an ice bath in the refrigerator overnight. Slowly pipette and mix the matrix gel with a pre-cooled pipette tip 5 times while being careful not to produce bubbles. If bubbles are produced, remove them by briefly spinning the mixture in a low-speed centrifuge.
2. Place the cell culture plate for preheating in an incubator.
3. Pipette the thawed matrix gel into aliquots using a pre-cooled pipette tip.
4. Dilute the Extragel with the pre-cooled serum-free medium at a ratio of 1:100. Spread the diluted matrix gel evenly and completely cover the cell culture plate. It is recommended to use 300 μ L/cm² of the diluted matrix gel for plate coating.
5. Let the coated cell culture plate stand at room temperature for one hour.
6. Remove the coating solution and immediately seed the stem cells with a mixture of culture medium. Be sure not to let the surface of the coated cell culture plate dry out.

APPLICATION CASES

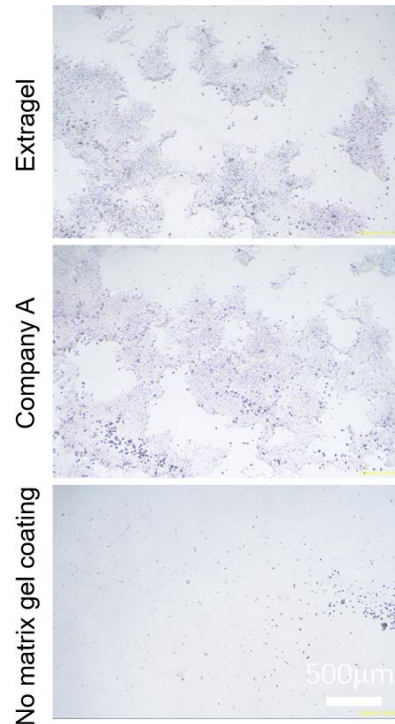


Figure 1. The growth of human embryonic stem cells after 2 days on Extrigel, the matrix gel of Company A, and uncoated cell culture plates.

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 the 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).

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