ECMatrix™-511 Silk E8 Laminin Substrate

Stem Cell Reagent
Cat. # CC161-100ML

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

Pack size: 100 mL

Store at 2-8°C

Data Sheet

Description

Human pluripotent stem cells (ES and iPS cells) express α6β1 as the major integrin species and therefore can be maintained stably and expanded efficiently in feeder-free conditions on culture vessels coated with it's binding partner laminin-511. However, laminin-511 is not suitable for large-scale production because of its large molecular weight and heterotrimeric nature. Professor Kiyotoshi Sekiguchi's group (Matrixome, Inc.) have solved this problem by producing a recombinant E8 fragment of laminin-511 at large-scale while retaining the full integrin binding activity.[1] The ECMatrix™-511 E8 Laminin Substrate can be used to culture pluripotent stem cells in feeder-free conditions with numerous added benefits over traditional methods including:

Features and Benefits

• **Animal-free, xeno-free format**: Consistent from lot-to-lot with no prescreening required

• **No plate precoating required**: Save time by simply adding to media while passaging cells

• **Supports single cell passaging w/out ROCKi**: Great for CRISPR editing or clonal isolation

• **Higher adhesion and growth rates**: Get to your experiments faster

• **Easy to handle**: No chilling of cell culture consumables required

Storage and Handling

ECMatrix™-511 E8 Laminin Substrates should be stored at 2-8°C. Avoid multiple freeze-thaw cycles and protect from light.

Presentation

1) 1 X 100 mL ECMatrix™-511 E8 Laminin Substrate (1.6 µg/mL in recombinant HSA/PBS). Expressed in transgenic silkworm cocoon.

Quality Control Testing

- Purity (SDS-Page): > 95%
- Endotoxin Test: ≤ 750 EU/mg
- Mycoplasma Test: Negative
- Sterility Test: Negative
- Integrin Binding Assay (kDa): ≤ 10 nM

Protocol

Depending on application, either a precoating or non-precoating method can be used to culture pluripotent stem cells.

Non-Precoating Method

1. Detach cells into small clumps or single cells using Accutase.
2. Add ECMatrix™-511 to fresh media at a final concentration of 0.25 ug/cm^2 (for example: for one well of a 6-well plate add 5 uL of the 0.5 mg/mL stock solution).
3. Add cells to the ECMatrix-511™/Media and plate the cells at desired density.

Precoating Method

1. Dilute the 0.5 mg/mL stock solution with sterile PBS to achieve a 2.5 ug/mL working solution.
2. Coat dishes with ECMatrix™-511 at 0.25 ug/cm^2 (for example, for one well of a 6-well plate add 1 mL of the 2.5 ug/mL working solution).
3. Incubate for 1 hour at 37°C, 3 hours at room temperate or overnight at 4°C.
4. Before use, remove remaining fluid from the coated surface (do not rinse).
5. Detach cells into small clumps using Accutase.
6. Plate the cells at desired density.

Note: Do not allow the plates to dry, briefly spin down all liquids in the tube before use, avoid repeated freeze-thaw cycles.

References

1. Sekiguchi K et al. Laminin E8 fragments support efficient adhesion and expansion of dissociated human pluripotent stem cells. Nature Commun. 2012;3:1236.
2. Yamanaka S, et al. A novel efficient feeder-free culture system for the derivation of human induced pluripotent stem cells. Sci Rep. 2014 Jan 8;4:3594.
3. Takashima Y, et al. Resetting transcription factor control circuitry toward ground-state pluripotency in human. Cell. 2014 Sep 11;158(6):1254-1269.
4. Miyazaki T, et al. Efficient Adhesion Culture of Human Pluripotent Stem Cells Using Laminin Fragments in an Uncoated Manner. Sci Rep. 2017 Jan 30;7:41165.

SPECIES LEGEND: **H** Human **Ca** Canine **M** Mouse **R** Rat **Rb** Rabbit **B** Bovine **P** Porcine **WR** Most Common Vertebrates

Ver 1.0/2019-08-12/CC161-100ML/NJA

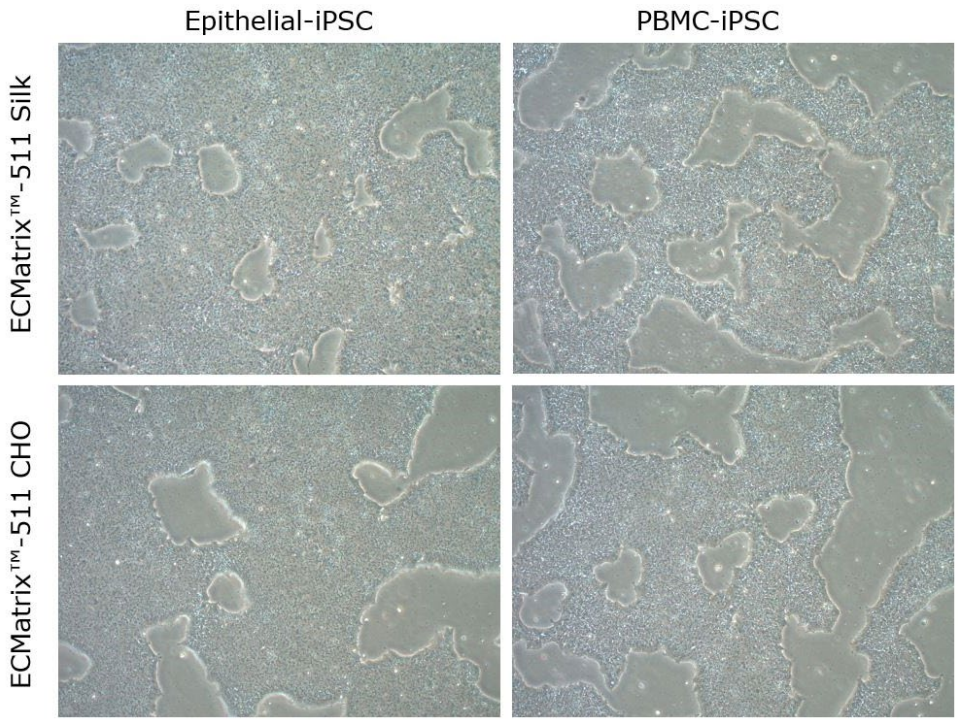

Figure 1. Growth of human induced pluripotent stem cells on Silk vs. CHO derived ECMatrix™-511 E8 Laminin Substrates. Epithelial or PBMC derived human iPSCs grown on silkworm or CHO derived ECMatrix™-511 E8 Laminin Substrates have similar proliferation rates and pluripotent morphologies over a 3-day culture period.

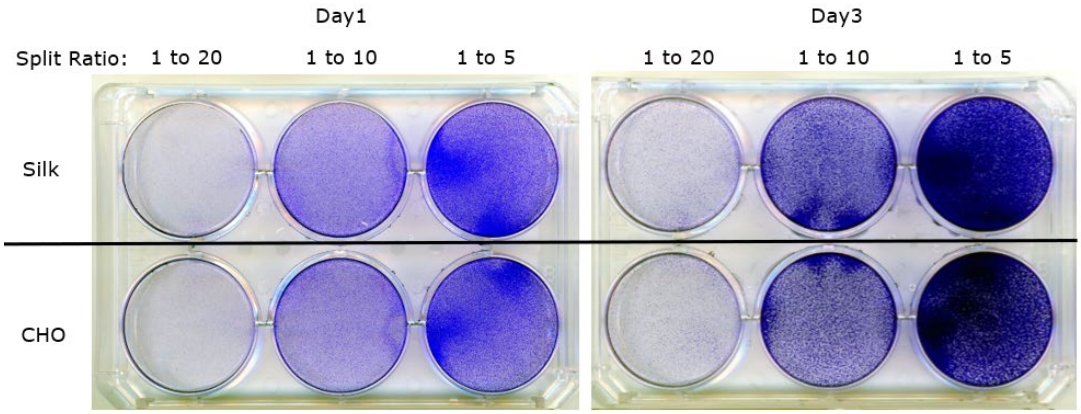

Figure 1. Crystal violet staining of human induced pluripotent stem cells. Epithelial derived human iPSCs passaged using a split ratio of 1:5 to 1:20 grown on silkworm or CHO derived ECMatrix™-511 E8 Laminin Substrates have similar proliferation rates over a 3-day culture period. Cells stained blue with crystal violet.