

ECMatrix™-511 Silk E8 Laminin Substrate

Stem Cell Reagent

Cat. # CC161-1050UG

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

Pack size: 1050 µg

Store at 2-8°C



Data Sheet

page 1 of 2

Description

Human pluripotent stem cells (ES and iPS cells) express $\alpha 6 \beta 1$ as the major integrin species and therefore can be maintained stably and expanded efficiently in feeder-free conditions on culture vessels coated with its 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 precoat 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) 6 X 175 µg ECMatrix™-511 E8 Laminin Substrate (0.5 mg/mL in 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 precoat or non-precoat method can be used to culture pluripotent stem cells.

Non-Precoat 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 µg/cm² (for example: for one well of a 6-well plate add 5 µL of the 0.5 mg/mL stock solution).
3. Add cells to the ECMatrix-511™/Media and plate the cells at desired density.

Precoat Method

1. Dilute the 0.5 mg/mL stock solution with sterile PBS to achieve a 2.5 µg/mL working solution.
2. Coat dishes with ECMatrix™-511 at 0.25 µg/cm² (for example, for one well of a 6-well plate add 1 mL of the 2.5 µg/mL working solution).
3. Incubate for 1 hour at 37°C, 3 hours at room temperature 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

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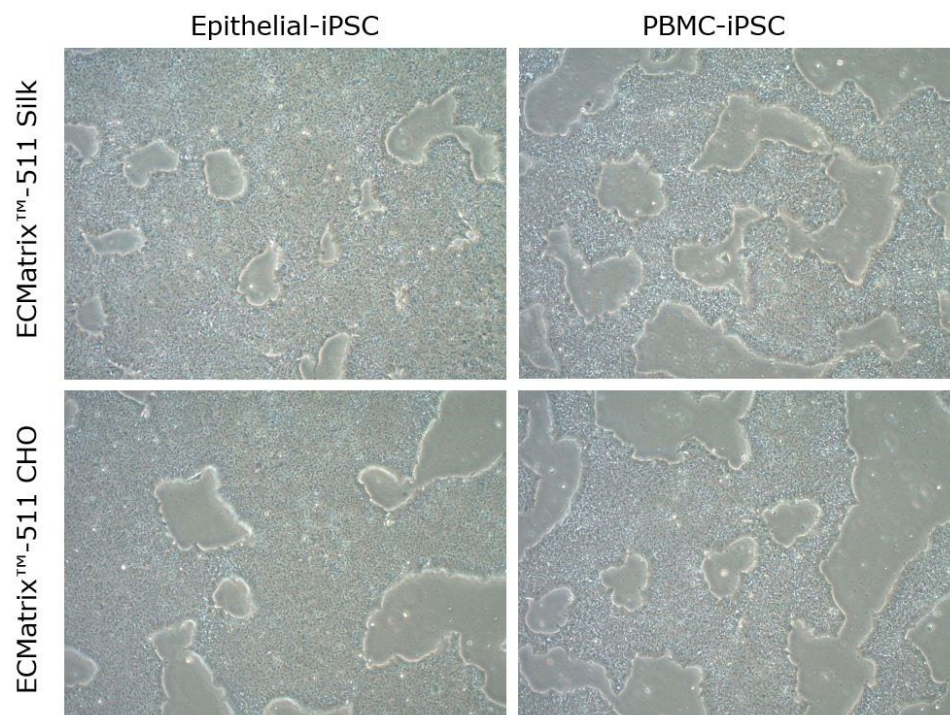


Figure 1. Growth of human induced pluripotent stem cells on Silk vs. CHO derived ECMatrix™-511 E8 Laminin Substrates. Epithelial or PBMC derived human iPSCs grown on silkworm or CHO derived ECMatrix™-511 E8 Laminin Substrates have similar proliferation rates and pluripotent morphologies over a 3-day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

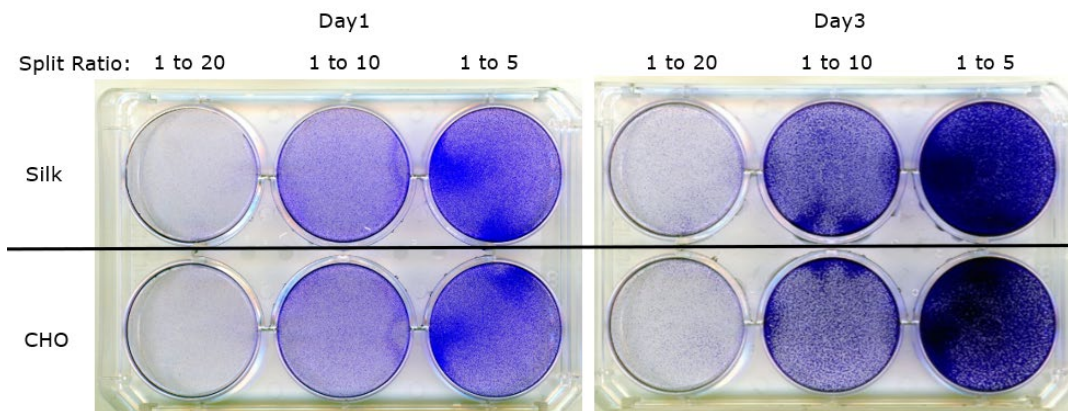


Figure 1. Crystal violet staining of human induced pluripotent stem cells. Epithelial derived human iPSCs passaged using a split ratio of 1:5 to 1:20 grown on silkworm or CHO derived ECMatrix™-511 E8 Laminin Substrates have similar proliferation rates over a 3-day culture period. Cells stained blue with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

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