ECMatrix™-511 E8 Laminin Substrate

Stem Cell Reagent
Cat. # CC160-350UG

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

Pack size: 350 µg

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) 2 X 175 µg ECMatrix™-511 E8 Laminin Substrate (0.5 mg/mL in PBS). Expressed in CHO-S cells.

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/CC160-350UG/NJA

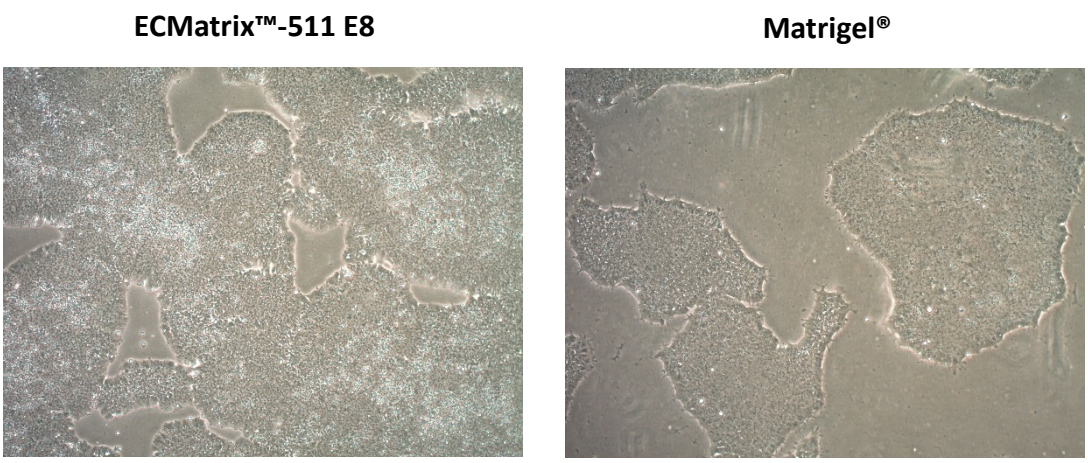
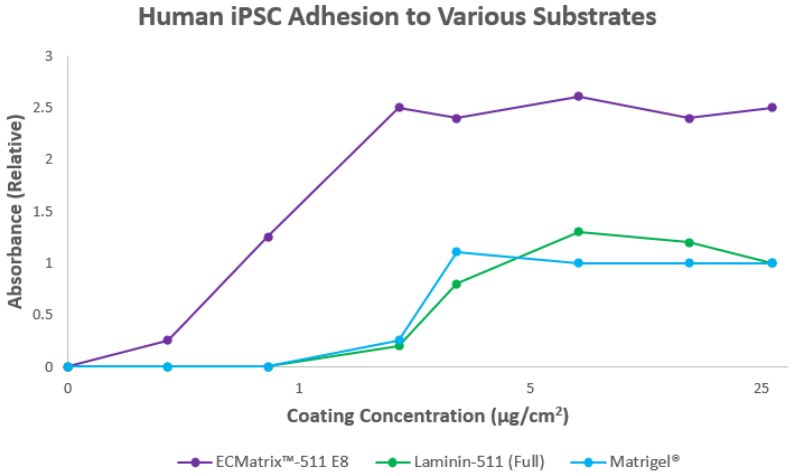


Figure 1. Feeder-free growth of human induced pluripotent stem cells. Human iPSCs grown on ECMatrix™-511 E8 Laminin Substrates demonstrate higher cell proliferation rates compared to Matrigel basement membrane extract when grown in feeder-free conditions and passaged as single cells using Accutase.



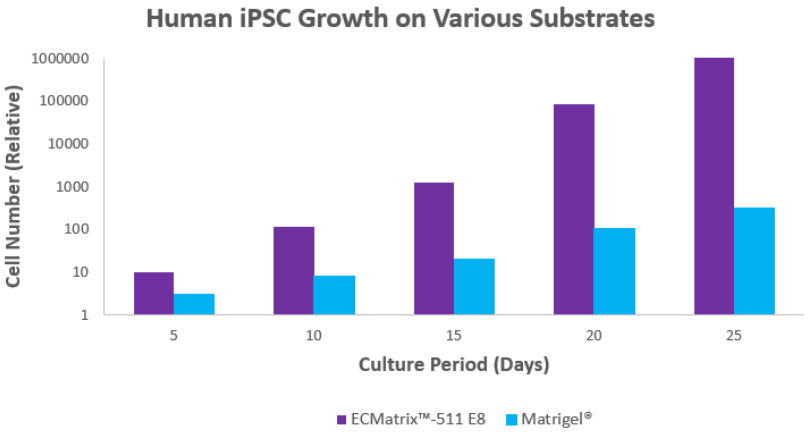


Figure 2. Adhesion and growth of human iPSCs on ECMatrix™-511 E8 Laminin Substrates. A) Human iPS cells adhere more strongly to ECMatrix™-511 E8 Laminin Substrates than to the intact Laminin-511 or Matrigel®. The absorbance (OD570) represents the relative number of attached cells normalized against the values at the maximum effect on Matrigel, which was arbitrarily set as 1. B) Human iPS cells cultured using ECMatrix™-511 E8 Laminin Substrates resulted in 200-fold more cells in comparison to Matrigel® coating methods over a 25-day culture period.