BioLamina
REVOLUTIONIZING CELL CULTURE

CULTURING ES AND iPS CELLS
ON HUMAN RECOMBINANT BIOLAMININ 511

INSTRUCTIONS FOR USE **BL002**

BIOLAMINA DEVELOPS, MANUFACTURES AND DISTRIBUTES LAMININ CELL CULTURE REAGENTS, BIOLAMININ MATRICES, THAT MAKES IT POSSIBLE TO CULTURE PRIMARY CELLS AND CELL LINES IN A CELL SPECIFIC AND BIOLOGICALLY RELEVANT ENVIRONMENT. LAMININ 511 IS A KEY PROTEIN OF THE NATURAL STEM CELL NICHE AND IS EXPRESSED IN THE DEVELOPING EMBRYO. THE DEFINED AND ANIMAL COMPONENT-FREE BIOLAMININ 511 (LN511) CELL CULTURE MATRIX CREATES A MORE AUTHENTIC CULTURE ENVIRONMENT THAT SUPPORTS ROBUST EXPANSION OF MOUSE PLURIPOTENT STEM CELL (mPSC) WHERE THE ADDITION OF DIFFERENTIATION INHIBITORS, SUCH AS LEUKEMIA INHIBITORY FACTOR (LIF), IS NO LONGER NEEDED. ICM CELLS CULTURED ON LN511 MAINTAIN NAIVE PLURIPOTENCY CAN BE USED FOR GERMLINE TRANSMISSION. FURTHERMORE, EASY AND CONTROLLED SINGLE-CELL PASSAGING CAN BE PERFORMED, TOTALLY INDEPENDENT OF ROCK INHIBITOR (ROCKi). LN511 ALSO SUPPORT CLONAL CULTIVATION.

IMPORTANT NOTES

- **All procedures should be done under sterile conditions using aseptic techniques**
- **The protocols can easily be made totally defined and animal component-free with your choice of culture medium and dissociation reagent**
- **It is important that the cells transitioned to the LN511 matrix are of high quality**
- **Some mPSC lines transitioned to the LN511 matrix might require an adaptation period before they can be cultured according to the single-cell passaging protocol**
- **Once adapted to the LN511 matrix, mPSCs can routinely be cultured as single cells without ROCKi**
- **The LN511 matrix facilitates long-term self-renewal of mPSC without the addition of LIF**

TRANSFER PROTOCOL

This is a protocol for the transition of mPSCs to the Biolaminin 511 (LN511) cell culture matrix from another feeder-free matrix (e.g. Matrigel) or from feeder cells.

1. Coat new cultureware with Biolaminin 511 as described in **INSTRUCTIONS FOR USE 001.**

2. For the transition step, split you cells as you normally do and then seed the cells on the Biolaminin 511 coated plate. So, if you are culturing your cells as colonies, transition to the Biolaminin 511 substrates as colonies.
   - *It is important that the cells transferred to the Biolaminin 511 matrix are of high quality. Carefully select only undifferentiated cell areas for transfer.*
   - *It is to be expected that cell morphology will look different on Biolaminin 511 compared to cells cultured as colonies on feeder or on other feeder-free matrices.*
   - *It is not recommended to change both the medium and matrix brand at the same time. Preferably, transition the mPSCs to the Biolaminin 511 matrix before undertaking a gradual medium transition.*

3. For the next passage, follow our protocol described in the passaging and culture instructions below. Some mPSC lines are more difficult to transition to the Biolaminin 511 matrix and might require an adaptation period (up to 5 passages) before they can be cultured as single-cells or small aggregates. To insure a successful transition, we recommend that you do the following:
   - add ROCK inhibitor (10 uM) for the first few passages
   - use a higher coating concentration (10 ug/ml)
   - seed at a higher cell density (50,000-100,000 cells/cm^2) for the first few passages

   Once the cells are adapted to the Biolaminin 511 matrix, the seeding density and coating concentration usually can be lowered and the hPSCs can routinely be cultured as single cells without need of ROCK inhibitor.

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PASSAGING PROTOCOL

The following protocol is for easy single cell passage of mPSCs on Biolaminin 511. This is a generic guideline that might require optimization for best results. Volumes given in this section are for 6-well plates and should be adjusted accordingly for different sized cultureware. Once successfully transferred and adapted to the LN511 matrix, mPSCs can be cultured as single cells without the addition of ROCKi or LIF.

BEFORE START:

- Coat new cultureware with the Biolaminin 511 cell culture matrix as described in **INSTRUCTIONS FOR USE 001**.
- Aliquot sufficient amounts of all solutions needed for the protocol and pre-warm to +37°C.

1. Carefully remove the excess Biolaminin coating solution from a new plate without disturbing the coated surface. Immediately add 2 mL fresh culture medium of choice to each well. Let equilibrate at +37°C, with 5% CO_2 and 95% humidity.
   - *Do not allow the coated surface to dehydrate as that will inactivate the Biolaminin coating.*
   - *Culture medium for different cell types and applications can be determined accordingly by the user. No LIF has to be added.*
2. Aspirate the medium from the cells and rinse gently with 1xDPBS (Ca^{--}/Mg^{--}) (1 mL/well).
   - *DPBS without Ca^{2+} and Mg^{2+} should be used since divalent cations have negative effect on some dissociating enzymes.*
3. Add 1 mL/well of dissociation reagent of choice (e.g. TrypLE™, EDTA, Accutase, Trypsin) and incubate at +37°C for 3-6 minutes.
   - *The incubation time is cell line and dissociation reagent dependent. Stem cells are sensitive and too much mechanical stress caused by extensive pipetting may result in low cell viability. A more confluent culture and the use of a high coating concentration could make the cells attach more tightly to the surface and a prolonged treatment time with the dissociation agent may be required. If it's still difficult to dissociate the cells, try lowering the coating concentration.*
4. Gently aspirate the cell dissociation solution and add 1-2 mL/well of pre-warmed fresh medium. Gently pipette up and down 2-4 times to achieve single-cell suspension. The mechanical force applied should be minimal not to cause significant physical damage to the cells.
   - *Use a microscope to verify that the cells are properly dissociated. When using BioLaminin 511, we recommend passaging pluripotent stem cells as single cells or as small aggregates. When using EDTA, try to achieve as small cell aggregates as possible without using too much mechanical force. Extensive pipetting may result in low cell viability. Rather, increase the incubation time to minimize pipetting.*
5. Collect the cell suspension in a 15 mL conical tube. Centrifuge at 100 x *g* for 4 minutes at room temperature (+15°C to +25°C).
6. Aspirate and discard the supernatant and gently resuspend the cell pellet in fresh, pre-warmed culture medium of choice. For each well of mPSCs collected, add 1-2 mL of medium.
7. Count the cells and calculate the cell suspension volume needed for seeding.
   - *Optimal seeding densities will vary from one cell line to another. The Biolaminin 511 culture system is flexible and the split ratio can be adjusted empirically for each protocol and need. Biolaminin 511 can support cell survival at a seeding density as low as 5,000 cells/cm^2.*
   - *When transitioning your cells to Biolaminin 511 from another feeder-free matrix (e.g. Matrigel) or from feeder cells, follow the instructions in the* **TRANSFER PROTOCOL** *above.*
8. Bring out the fresh BioLaminin 511 coated tissue culture plate containing fresh, pre-warmed culture medium and transfer the desired volume of cell suspension to each well. Immediately rock the plate to get an even cell distribution.
   - *If you are transitioning your cells to BioLaminin 511 from feeders or another feeder-free matrix, we recommend adding ROCK inhibitor to a final concentration of 10 uM for the first few passages. Once adapted to the BioLaminin matrix, the cells can routinely be cultured as single cells without the need for ROCK inhibitor.*
9. Place the plate into the incubator and culture the cells at +37°C, with 5% CO_2 and 95% humidity. Perform a complete medium change 24-48 hours after passaging and then feed cells on daily basis until the next passage. For reduced labor and cost, follow the weekend-free protocol described in APPLICATION NOTE 001.
   - *mPSCs cultured on LN511 should grow as a homogenous monolayer, without any differentiated areas.*

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