BioLamina
REVOLUTIONIZING CELL CULTURE

CULTURING ES AND iPS CELLS
ON HUMAN RECOMBINANT LAMININ-511

INSTRUCTIONS FOR USE **BL002**

BIOLAMINA DEVELOPS, MANUFACTURES AND DISTRIBUTES LAMININ (LN) CELL CULTURE REAGENTS THAT MAKES IT POSSIBLE TO CULTURE PRIMARY CELLS AND CELL LINES IN A CELL SPECIFIC AND BIOLOGICALLY RELEVANT ENVIRONMENT. LN-511 IS A KEY PROTEIN OF THE NATURAL STEM CELL NICHE AND IS EXPRESSED IN THE DEVELOPING EMBRYO. THE DEFINED AND XENO-FREE LN-511 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 LN-511 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). LN-511 ALSO SUPPORT CLONAL CULTIVATION. THE CELLS GROW IN A HOMOGENEOUS MONOLAYER WITHOUT NEED FOR MANUAL REMOVAL OF DIFFERENTIATED CELL AREAS.

IMPORTANT NOTES

- **All procedures should be done under sterile conditions using aseptic techniques**
- **The protocols can easily be made totally defined and xeno-free with your choice of culture medium and dissociation reagent**
- **It is important that the cells transferred to the LN-511 matrix are of high quality**
- **Some mPSC lines transferred to the LN-511 matrix, might require an adaptation period before they can be cultured according to the single-cell passaging protocol**
- **Once adapted to the LN-511 matrix, mPSCs can routinely be cultured as single cells without ROCKi**
- **The LN-511 matrix facilitates long-term self-renewal of mPSC without the addition of LIF.**

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TRANSFER PROTOCOL

The transfer of mPSCs from another feeder-free matrix (e.g. Matrige) or from feeders to the LN-511 cell culture matrix is often quite straight forward. Coat a new plate with the LN-511 matrix according to **INSTRUCTIONS FOR USE 001** and perform single-cell passage as described in the **PASSAGING PROTOCOL** below. It is to be expected that cell/colony morphology will look different when compared to cells grown using other culture conditions.

- *It is important that the cells transferred to the LN-511 matrix are of high quality. Carefully select only undifferentiated cell areas for transfer. Initially, it's recommended to seed 2-3 wells of a smaller well format (e.g. 48-well format), for the option to choose a well with most homogeneous cell population for further use.*
- *Use a higher seeding density (50,000-100,000 cells/cm^2) for the first number of passages.*
- *It is not recommended to change both medium and matrix brand at the same time. Preferably transfer the mPSCs to the LN-511 matrix before doing the medium transition the next day.*

Some mPSC lines are more difficult to transfer and might require an adaptation period before they can be cultured as single cell as described in the **PASSAGING PROTOCOL** described below. When the cell transfer to the LN-511 matrix is problematic, try the following:

1. Increase the coating concentration to 10 ug/mL. Once the cells are adapted, a lower coating concentration can often be used which should be optimized empirically for each cell line.
2. Transfer and culture the cells as small aggregates or as single cells in combination with ROCKi for a few passages. Once adapted, mPSCs can routinely be cultured as single cells without ROCKi, as described below. It may take up to 5 passages for some cell lines become acclimatized to LN-511.

PASSAGING PROTOCOL

The following protocol is for easy single cell passage of mPSCs on LN-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 LN-511 matrix, mPSCs can be cultured as single cells without the addition of ROCKi.

BEFORE START:

- Coat new cultureware with the LN-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 laminin 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 laminin 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™ or EDTA) and incubate at +37°C for 3-6 minutes.
   - *The incubation time is cell line and dissociation reagent dependant. It also depends on the laminin coating concentration used and the degree of cell confluence. More confluent cultures might need longer treatment time compared to sub-confluent cultures. However, stem cells are sensitive and too long exposure to enzymes or too much mechanical stress caused by extensive pipetting may result in low cell viability. If it's still difficult to dissociate the cells, try lowering the laminin coating concentration.*
4. Gently aspirate the dissociation solution and add 1-2 mL/well of pre-warmed fresh medium. Gently pipette up and down 4 - 8 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. It's harder to get the cells into a single cell suspension using EDTA compared to enzymatic dissociation. 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 flick the tube to fully dislodge the cell pellet from the tube bottom. 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 cell number and plate the cells onto the LN-511 coated plate prepared in step 1.
   - *Optimal seeding densities will vary from one cell line to another. The LN-511 culture system is flexible and the split ratio can be adjusted empirically for each protocol and need.*
   - *When transferring your cells from another feeder-free matrix (e.g. Matrigel) or from feeder cells, follow the instructions in the* **TRANSFER PROTOCOL** *above.*
8. Place the plate into the incubator and gently rock the plate to distribute the cells evenly.
9. Culture the cells at +37°C, with 5% CO_2 and 95% humidity. Feed cells on daily basis until next passage. Freshly seeded cells only need a few drops of fresh medium after 24 hours. Perform a complete medium change 48 hours after passaging.
   - *Within 60 minutes, the majority of the cells should have attached, evenly distributed as single cells across the well. The day after seeding the cells should have formed small colonies.*
   - *mPSCs cultured on LN-511 should grow as a homogenous monolayer, without any differentiated areas.*

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