BioLamina
REVOLUTIONIZING CELL CULTURE

CLONEstem-To-Go™
GENERATION OF STEM CELL CLONES

INSTRUCTIONS FOR USE **BL005**

CLONEstem-To-Go™ IS A PRE-COATED PLATE FOR EASY AND EFFICIENT GENERATION OF GENETICALLY STABLE HUMAN PLURIPOTENT STEM CELL CLONES

All procedures should be done under sterile conditions using aseptic techniques. Before start, all solutions used for cell passaging should be aliquoted in sufficient amounts and pre-warmed at +37°C.

CLONE GENERATION

- Prepare the clonal medium (CM) by adding 1 mL of 20% albumin solution to 9 mL of your PSC culture media of choice and pre-warm.
  Culture medium and albumin solution should be determined accordingly by the user for different cell types and applications. We have successfully tested bovine albumin (Sigma B4287, dissolved in DMEM/F12 w/o phenol red) and human plasma albumin (Albuminativ 20%, Octopharma) but the performance between different brands and batches might vary.
- Wipe down the outside of the aluminum bag containing the pre-coated plate with 70% ethanol to sterilize before opening.
  Make sure the foil wrapping has not been punctured as this may have inactivated the Laminin coating.
- Wash the wells twice with 80 uL of 1 x DPBS buffer (Ca^{++}/Mg^{++}).
  Note that the DPBS for washing must contain Ca^{2+} and Mg^{2+} since divalent cations are important for the protein structure and function.

  Do not let the Laminin wells dry out at any point of procedure, as this will inactivate the Laminin matrix.
- Add 50 µL of complete clonal medium to each well and let equilibrate at +37°C, 5% CO_2.
- Passage your PSC into single-cell suspension as described in **INSTRUCTIONS FOR USE BL003**.
  Best results are obtained if your cells have been adapted and cultured on LN-521™ before.

  If using FACS sorting, seed one cell in each well, otherwise follow the dilution protocol below where the cell suspension is diluted to 200 cells/mL, which theoretically results in 0-4 cells/well.

IMPORTANT NOTES

- **The protocol can easily be made totally defined and xeno-free with your choice of culture medium and enzyme**
- **CLONEstem-To-Go™ pre-coated plates maintain functionality for at least 9 months when stored at room temperature (+15°C to +25°C)**
- **The following protocol is a generic guideline that might require optimization for best results**
- **No treatment with artificial apoptosis inhibitors, such as Rho-kinase (ROCK) inhibitor is needed**

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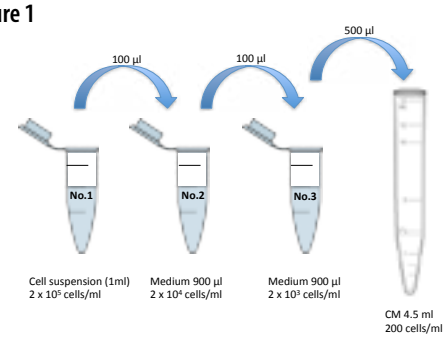
- Count the cell number and adjust the number of cells to 2 x 10^5 cells/mL by taking out 2x 10^5 cells and add to pre-warmed medium of choice for a total volume of 1mL (tube No.1 in Figure 1 below). Prepare the tubes for the dilution series by adding correct amount medium to each of them. Make a dilution series with your medium of choice and dilute cells in CM for the final cell suspension of 200 cells/mL as described in figure 1 below.

Figure 1



- Add 20 µL of the final cell suspension to each well containing pre-warmed clonal medium. Make sure to mix regularly for a homogenous cell suspension.
  Tip: *Use higher cell concentration in one control well (e.g. 15 000 cells) in order to easier find the focal plane of the single cells with the microscope.*
- Incubate the plate at +37°C, 5% CO_2 for 48 hours without disturbing the plate.
- After 48 hours, change medium every day by replacing old medium with 100 µL/well of your cell culture medium of choice for a few more days.
- Using a microscope, carefully inspect at day 6 the earliest the emerging single cell-derived colonies.
  Do not keep the plate too long in room temperature since the cells are sensitive to temperature changes.
  Tip: *A quick look at the control well can be done already at 24 hours after seeding. The cells should be well attached and have formed small colonies.*

CLONE EXPANSION

The cells are ready to be passaged when the colony covers 30-50% of the well. A good colony that originates from a single cell is usually ready to be passaged 9-12 days post-seeding, however, the time may vary between different cell lines.

- Coat new cultureware in advance with LN-521™ coating solution as described in **INSTRUCTIONS FOR USE BL001** or use 521-To-Go™ pre-coated plates (**INSTRUCTIONS FOR USE BL007**).
  We recommend transferring the cells to a smaller plate format (e.g. 48-well or 24-well plate format) for the first passage from CLONEstem-To-Go™ plate.
- Perform a conventional single-cell passage on LN-521™ as described in **INSTRUCTIONS FOR USE BL003**. Transfer the cells from each well to new freshly coated wells.
  Seed a higher cell density for the first number of passages (50,000–100.000 cells/cm²) before lowering the seeding density to best fit your cells and application.

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