LaminStem

LaminStem facilitates self-renewal of both pluripotent human embryonic stem cells and induced pluripotent stem cells in a chemically defined, feeder-free and xeno-free cell culture system. Importantly, LaminStem allows the survival and expansion of human ES and iPS cells after plating from single cell suspension. LaminStem based stem cells grow as monolayers on top of the laminin substrate and remain pluripotent without spontaneous differentiation.

Cat. No.: 05-753-1

100µg/1ml

Store at: -20°C

Instructions for Use

Coating Procedure

- Slowly thaw LaminStem at +2-8°C before use.
- Dilute the thawed LaminStem stock solution with 1xDPBS containing Ca^{++} and Mg^{++} (see tables).
- Add the diluted LaminStem solution to tissue culture-treated cultureware for a final coating concentration of 0.5-2 ug/cm^2. The optimal coating concentration is cell-dependent. Please see tables bellow.
- Seal the plate (e.g. with Parafilm®) to prevent evaporation and incubate at +2°C to +8°C overnight. If a more rapid coating is required, incubate at +37°C for 2 hours. Make sure the LaminStem solution is spread evenly across the surface. Note that the LaminStem matrix will be inactivated if let dry.

Storage and Stability

The LaminStem stock solution is stable for 2 years when stored at -20°C. Expiry date on label.

- If desired, the LaminStem stock can be dispensed into working aliquots and stored at -20°C.
- Repeated freeze thawing should be avoided.
- Thawed LaminStem stock is stable for at least 3 months when stored at +2°C to +8°C under aseptic conditions.
- For your convenience, the coated plates and diluted coating solution can be kept for up to 4 weeks when stored aseptically at +2°C to +8°C.

Important Notes

- When using LaminStem no treatment with apoptosis inhibitors, such as ROCK or blebbistatin, is needed.
- The Procedure can easily be made totally defined and xeno-free with your choice of culture medium and enzyme.
- Before start, all solutions used for cell passaging should be aliquoted in sufficient amounts and pre-warmed at +37°C, 5% CO_2.
- Cells are ready to be passaged when cell culture is $\geq$60% confluent. Optimal seeding densities will vary from one cell line to another and can be determined empirically for your system. With optimal medium conditions and seeding density, most cell lines will reach confluence within 4-6 days and expand 10-25 fold.

Recommended Coating Concentration

First time use

When using the LaminStem matrix for the first time the cells might need some adaptation, hence a higher coating concentration is recommended for the first few passages. See table 1 below for recommended volumes and concentrations.

Table 1

Cultureware	Surface area cm^2	Coating concentration (ug/ml)	Coating concentration (ug/cm^2)	Laminstem stock volume	1XDPBS (ca++/mg++) volume	Total coating volume
6-well	9.6	10	1.0	100 uL/well	900 uL/well	1 mL/well
12-well	3.9	10	1.25	50 uL/well	450 uL/well	500 uL/well
24-well	1.9	10	1.5	30 uL/well	270 uL/well	300 uL/well
48-well	0.75	10	1.75	17.5 uL/well	157.5 uL/well	175 uL/well
96-well	0.34	10	2.0	6 uL/well	54 uL/well	60 uL/well
T-25cm^2 flask	15	10	1.0	250 uL/well	2.250 mL/Flask	2 .5 mL/flask
T-75cm^2 flask	75	10	1.0	750 uL/well	6.750 mL/Flask	7.5 mL/flask

Note:*
Please note that the coating concentration/cm^2 is higher for smaller culture surfaces due to higher surface tension.

Routine use

Once the cells are adapted to the LaminStem matrix a lower coating concentration can usually be used. See table 2 for recommended coating volumes and concentrations.

Table 2

Cultureware	Surface area cm^2	Coating concentration (ug/ml)	Coating concentration (ug/cm^2)	Laminin stock volume	1Xdpbs (ca++/mg++) volume	Total coating volume
6-well	9.6	5	0.5	50 uL/well	950 uL/well	1 mL/well
12-well	3.9	5	1.625	25 uL/well	475 uL/well	500 uL/well
24-well	1.9	5	0.75	15 uL/well	285 uL/well	300 uL/well
48-well	0.75	5	0.875	8.75 uL/well	131.25 uL/well	140 uL/well
96-well	0.34	5	1.0	3 uL/well	57 uL/well	60 uL/well
T-25cm^2 flask	15	5	0.5	125 uL/well	2.375 mL/Flask	2 .5 mL/flask
T-75cm^2 flask	75	5	0.5	375 uL/well	7.125 mL/Flask	7.5 mL/flask

Note:
* Please note that the coating concentration/cm^2 is higher for smaller culture surfaces due to higher surface tension.

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Culture of Excellence

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