

LaminStem[™] 521

Human Recombinant Laminin Defined matrix for pluripotent stem cell culture

REF 05-753-1F

-20°C to -80°C

Volume: 1ml

Concentration: 0.1mg/ml

Instructions for Use

Product Description

LaminStem[™] facilitates self-renewal of both pluripotent human embryonic stem cells and induced pluripotent stem cells in a defined and feeder-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.

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 on page 2).
- Add the diluted LaminStem[™] solution to tissue culturetreated cultureware for a final coating concentration of 0.5-2 ug/cm². The optimal coating concentration is cell-dependent. (Please see tables on page 2).
- 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 to -80°C. Expiry date on label.
- If desired, the LaminStem[™] stock can be dispensed into working aliquots and stored at -20°C to -80°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.
- 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 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% CO₂.
- Cells are ready to be passaged when cell culture is ≥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 (µg/cm²)	Laminstem stock volume	1XDPBS (ca++/mg++) volume	Total coating volume
6-well	9.6	1.0	96 µL/well	904 µL/well	1 mL/well
12-well	3.9	1.0	48 µL/well	452 µL/well	500 µL/well
24-well	1.9	1.0	19 µL/well	281 µL/well	300 µL/well
48-well	0.75	1.0	7.5 µL/well	167.5 µL/well	175 µL/well
96-well	0.34	1.0	3.4 µL/well	56.6 µL/well	60 µL/well
T–25cm² flask	25	1.0	250 µL/well	2.250 mL/flask	2.5 mL/flask
T–75cm² flask	75	1.0	750 µL/well	6.750 mL/flask	7.5 mL/flask

Note: Please note that the coating concentration/cm² 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 (µg/cm²)	Laminstem stock volume	1XDPBS (ca++/mg++) volume	Total coating volume
6-well	9.6	0.5	48 µL/well	952 µL/well	1 mL/well
12-well	3.9	0.5	24 µL/well	476 μL/well	500 µL/well
24-well	1.9	0.5	9.5 µL/well	290.5 µL/well	300 µL/well
48-well	0.75	0.5	3.75 µL/well	136.25 µL/well	140 µL/well
96-well	0.34	0.5	1.7 μL/well	58.3 µL/well	60 µL/well
T25-cm² flask	25	0.5	125 µL/well	2.375 mL/flask	2.5 mL/flask
T75-cm² flask	75	0.5	375 µL/well	7.125 mL/flask	7.5 mL/flask

Note: Please note that the coating concentration/cm² is higher for smaller culture surfaces due to higher surface tension.

Quality Assurance

For research use only.

Product Label Symbols



Indicates the need for the user to consult the instructions for use.

Legal

LaminStem[™] is manufactured by BioLamina.