Instructions for Use

LaminStem™ 521

Human Recombinant Laminin
Defined Matrix for Pluripotent Stem Cell Culture

2740200-000-00

SARTORIUS

Human Recombinant Laminin
Defined Matrix for Pluripotent Stem Cell Culture

REF	05-753-1F
	-20°C to -80°C
Volume	1 mL
Concentration	0.1 mg/mL

Contents

1 Product Description

LaminStem™ 521 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™ 521 allows the survival and expansion of human ES and iPS cells after plating from single cell suspension. LaminStem™ 521 based stem cells grow as monolayers on top of the laminin substrate and remain pluripotent without spontaneous differentiation.

2 Storage and Stability

- The LaminStem™ 521 stock solution is stable for 2 years when stored at -20°C to -80°C. Expiry date on label.
- If desired, the LaminStem™ 521 stock can be dispensed into working aliquots and stored at -20°C to -80°C.
- Repeated freeze thawing should be avoided.
- Thawed LaminStem™ 521 stock is stable for at least 3 months when stored at 2 – 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 – 8°C.

3 Important Notes

- When using LaminStem™ 521, no treatment with apoptosis inhibitors, such as ROCK or blebbistatin, is needed.
- 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 ≥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.

4 Coating Procedure

1. Slowly thaw LaminStem™ 521 at 2 – 8°C before use.
2. Dilute the thawed LaminStem™ 521 stock solution with 1xDPBS containing Ca++ and Mg++ (see tables on page 6).
3. Add the diluted LaminStem™ 521 solution to tissue culture-treated cultureware for a final coating concentration of 0.5 – 2 ug/cm². The optimal coating concentration is cell-dependent. (See tables on page 6).
4. Seal the plate (e.g. with Parafilm®) to prevent evaporation and incubate at 2 – 8°C overnight. If a more rapid coating is required, incubate at 37°C for 2 hours. Make sure the LaminStem™ 521 solution is spread evenly across the surface.

   NOTE that the LaminStem™ 521 matrix will be inactivated if let dry.

5 Recommended Coating Concentration

First time use
When using the LaminStem™ 521 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 on page 6 for recommended volumes and concentrations.

Table 1

Culture-ware	Surface area [cm^2]	Coating concen-tration [µg/ cm^2]	LaminStem™ 521 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
T25 Flask	25	1.0	250 µL/well	2.250 mL/flask	2.5 mL/flask
T75 Flask	75	1.0	750 µL/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™ 521 matrix a lower coating concentration can usually be used. See table 2 for recommended coating volumes and concentrations.

Table 2

Culture-ware	Surface area [cm^2]	Coating concen-tration [µg/ cm^2]	LaminStem™ 521 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 Flask	25	0.5	125 µL/well	2.375 mL/flask	2.5 mL/flask
T75 Flask	75	0.5	375 µL/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.

6 Quality Control

LaminStem™ 521 performance is tested for concentration, endotoxins, purity, mycoplasma and authentity. For full specifications please check the lot specific Certificate of Analysis (CoA).

7 Quality Assurance

For research use only.

7.1 Product Label Symbols

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

8 Legal

LaminStem™ 521 is manufactured by BioLamina AB, Löfströms allé 5a, 172 66 Sundbyberg, Sweden.

Sartorius Stedim Biotech GmbH
August-Spindler-Strasse 11
37079 Goettingen, Germany

Phone: +49 551 308 0
www.sartorius.com

The information and figures contained in these instructions correspond to the version date specified below.
Sartorius reserves the right to make changes to the technology, features, specifications and design of the equipment without notice.
Masculine or feminine forms are used to facilitate legibility in these instructions and always simultaneously denote all genders.

Last updated:

12 | 2021

MB | DIR: 2740200-000-00
Revision 01