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Laminin

From Engelbreth-Holm-Swarm (EHS) sarcoma (mouse)
Solution, filtered through 0.2 μm pore size membrane

Cat. No. 11 243 217 001

1 mg (2 ml)

Version 16
Content version: November 2015

Store at −15 to −25°C

1. What this Product Does

Contents

Solution, 0.5 mg/ml laminin in 0.15 M NaCl, 2 mM EDTA, 0.05 M Tris-HCl, pH 7.4, filtered through 0.2 μm pore size membrane.

Storage and Stability

The solution is stable at −15 to −25°C until the expiration date printed on the label. The undiluted solution is stable at +2 to +8°C for at least 3 months.

⚠ The solution should be carefully thawed at +15 to +25°C, do not freeze again.

Application

Laminin has been demonstrated to promote the attachment and growth of a variety of cells, *e.g.*,

- human carcinoma and sarcoma cells (1, 2),
- human retinoblastoma cells (3),
- liver cells (4),
- murine neuroblastoma cells (5),
- murine embryonal carcinoma cells (6)
- and seems to be involved in the development of embryonal tissue (7–10).

2. How to Use this Product

Working Concentration

For the coating of cell culture vessels 2 – 5 μg/cm^2 is used.

Additional Reagents Required

- 0.5 mg/ml Laminin, solution in 0.15 M NaCl, 2 mM EDTA, 0.05 M Tris-HCl, pH 7.4, stored at −15 to −25°C.
- Cell culture medium.

After thawing, the Laminin solution may contain some visible fibers. The presence of visible fibers does not have any effect on the behavior or function of the laminin in the cell culture.

Protocol

The following working instruction describes, as an example, the procedure for the coating of cell culture vessels with Laminin.

1. Thaw Laminin solution carefully at +15 to +25°C.
2. Take the required amount under sterile conditions and store laminin solution at +2 to +8°C.
   ⚠ Do not refreeze!
3. Prepare working dilutions with basal medium (without serum), *e.g.,* 20 μg/ml, if coating with 2 μg/cm^2 is required (dilute 40 μl laminin solution, 0.5 mg/ml, with 960 μl basal medium) or 50 μg/ml, if coating with 5 μg/cm^2 is required (dilute 100 μl laminin solution, 0.5 mg/ml, with 900 μl basal medium).
4. For each cm^2 surface to be coated add 100 μl diluted laminin solution, *e.g.*, to a 35 mm dish (10 cm^2) 1 ml is added.
5. Incubate vessels for 45 min in an incubator and then add cells suspended in culture medium in the desired plating density. Alternatively the diluted laminin solution may be aspired and then the cell suspension added. In this case the working dilution may be prepared with PBS.
6. Attachment and spreading of the cells can be monitored under a microscope [*e.g.,* for HT 1080 cells (human fibrosarcoma cells) after 30 min].
   ⚠ The optimal laminin concentration may vary with the cell type and has to be determined experimentally.

3. Additional Information on this Product

Description

Laminin is the major non-collagenous glycoprotein of basement membranes (11, 12). It is composed of two B-chains (B1: 230 kDa, B2: 220 kDa) and one A-chain (400 kDa) held together by disulphide bonds forming a large cross-shaped molecule (11).

- Laminin seems to be responsible for many kinds of cell-basement membrane interactions such as
  - adhesion,
  - migration, or
  - proliferation (12).
- It promotes the adhesion of many epithelial cell types to type IV collagen, including normal as well as tumor cells (13) but also of other cells bearing basement membranes such as myoblasts (14) or Schwann cells (15).
- In cell culture, laminin induces morphologic changes, for example
  - cell spreading (10, 16),
  - elongation (15, 17) or
  - neurite outgrowth (18–25)
- and serves as an attachment factor for epithelial and endothelial cells (14, 18).

Isolation and Properties

Laminin is purified as laminin-nidogen complex from mouse Engelbreth-Holm-Swarm (EHS) sarcoma (11, 21) according to the method of Timpl et al. (11, 10).

Primary Structure

Mouse laminin is composed of three polypeptide chains (A: 400 kDa, B1: 230 kDa and B2: 220 kDa), connected by disulphide bridges, and is glycosylated (26).

Molecular Weight

900 kDa

Quality

Purity: Laminin (as laminin-nidogen complex, 1:1) is >90% pure as determined by SDS-PAGE (27, 13).

Biological Activity

Tested for the promotion of adherence of HT-1080 cells (human fibrosarcoma cells).

Species Specificity

Mouse laminin is active on most mammalian cells *e.g.*, human, mouse, rat, rabbit, and also on *e.g.*, chicken and fish cells.

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4. Supplementary Information

4.1 Text Conventions

To make information consistent and memorable, the following text conventions are used in this package insert:

Text Convention	Use
Numbered Instructions labeled ❶, ❷,etc.	Steps in a procedure that must be performed in the order listed
Asterisk *	Denotes a product available from Roche Applied Science

Symbols

In this package insert the following symbols are used to highlight important information:

Symbol	Description
⚠	Important Note: Information critical to the success of the procedure or use of the product.

4.2 Changes to Previous Version

- Add information note regarding possible presence of visible fibers to Chapter 2, "How to Use this Product".
- Removed Chapter 4.3, Ordering Information
- Distributed by Sigma-Aldrich®

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Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany