

Certificate of Analysis

HIGH CONCENTRATION LAMININ/ENTACTIN COMPLEX

The laminin/entactin complex is a major component of the basement membrane in EHS mouse tumors. When purified from this source, laminin and entactin are present in an equimolar ratio.¹ Divalent cations have been shown to promote the formation of laminin/entactin aggregates.² At a concentration of approximately 2 mg/ml, the laminin/entactin complex can be used to form a three dimensional (3D) gel for studying cell growth and differentiation. The gelation of this material occurs in a temperature dependent manner.³ Since cellular differentiation is promoted by interactions between cells and simple or complex malleable extracellular matrix environments⁴, the culturing of cells in or on simple gels such as the laminin/entactin complex will enable the study of specific mechanisms that dictate cell differentiation and functionality. The laminin/entactin complex provides a 3D environment that more closely models the cellular microenvironment in vivo.⁵ Furthermore, high concentrations of laminin (2-6 mg/ml) have been used to study acinar differentiation of a human submandibular gland cell line^{6,7} and endothelial cell tubulogenesis.⁵

CATALOG NUMBER: 354259 LOT NUMBER: _____

SOURCE: Engelbreth-Holm-Swarm mouse tumor

QUANTITY: 10.5 mg, at _____ milligram per milliliter, frozen.

FORMULATION: Dulbecco's Phosphate-Buffered Saline

PURITY: ≥90 % by SDS-PAGE

USE: High Concentration Laminin/Entactin Complex will be used as a 3D matrix for cell differentiation assays. It will provide a highly defined 3D culture environment.

QUALITY CONTROL: The biological activity of the laminin/entactin complex is determined in a cell culture assay. NG-108 (mouse neuroblastoma/rat glioma) cells differentiated and formed neurites when plated on this lot of laminin/entactin complex.

Laminin/entactin is a membrane filtered (0.2µm) preparation, and is tested and found negative for the presence of bacteria, fungi and mycoplasma.

STORAGE: Stable when stored at -70°C. Avoid multiple freeze-thaws. Do not store in frost-free freezer. **KEEP FROZEN.**

EXPIRATION DATE:

REFERENCES:

1. Paulsson, M., et.al., Eur. J. Biochem., **166**:11(1987).
2. Paulsson, M., J. Biol. Chem., **263**:5425 (1988).
3. Yurchenco, P.D., et.al., J. Biol. Chem., **265**:3981 (1990).
4. Kleinman, H.K., et.al., Curr. Opin. Biotech., **14**:526 (2003).
5. Grant, D.S., et.al, Cell, **58**:933 (1989).
6. Hoffman, M.P., et.al., J. Biol. Chem., **273**:28633 (1998).
7. Zheng, C., et.al., J. Cell. Physiol., **177**:628 (1998).

Suggested Coating Procedures

CAUTION:

1. Do not allow this product to warm up above 4°C during manipulation. Keep the product on ice and dilute using ice-cold solutions or cell suspensions.
2. Because of the high viscosity always use a chilled syringe style displacement pipette such as the Gilson M series; avoid using air displacement pipettes.

GELATION PROCEDURE: Cells cultured on top of gel

1. Thaw the laminin/entactin concentrate slowly on ice. Keep the concentrate on ice at all times.
2. Dilute to the desired concentration: for a firm gel a concentration of at least 3.5 mg/ml is recommended.
3. Dilute using ice-cold isotonic salt solutions or media containing 0.1mM calcium.
4. Deliver the diluted laminin/entactin into the tissue culture plate and allow 1-2 hours at 37° to polymerize.

GELATION PROCEDURE: Cells cultured within the gel

1. Thaw as above.
2. Calculate the volume necessary to dilute the laminin/entactin to the desired concentration of at least 3.5 mg/ml. The dilution can be done directly in a chilled culture plate or in a chilled tube.
3. Add the chilled cell suspension to the concentrate followed by enough isotonic salt solution or media to bring the volume of cells plus media to that calculated in step 2.
4. Mix by gently drawing up the laminin/entactin-cell suspension into a Gilson M series pipette and expelling.
5. If the dilutions were done in a tube, pipette the suspension into the wells of a chilled culture plate and then incubate at 37°C for 1-2 hours to polymerize.

Quality Assurance

Date