
Certificate of Analysis

ECL Cell Attachment Matrix

(entactin-collagen IV-laminin)

Catalog # 08-110

Lot # 0701049396

Source: Engelbreth-Holm-Swarm (EHS) mouse tumor.

Use and Handling: Thaw frozen product at 4°C and keep on ice thereafter. Dilute with sterile serum-free medium to approximately 20 µg/ml. Add directly to culture vessels to achieve 5-10 µg/cm². Allow the matrix proteins to adsorb to the vessel for one hour at 37°C or overnight at 4°C prior to adding a cell suspension.

Sterility: This lot of ECL was tested and found negative for the presence of bacteria, fungi and mycoplasma.

Formulation: 5 mg in 5 ml of 0.05 M Tris-HCl, pH 7.4, 0.15 M NaCl. Protein determined by the Bradford dye binding assay using gamma globulin as the standard. Frozen Solution.

Storage and Stability: Stable for 1 year at -20°C from date of shipment. Aliquot to avoid repeated freezing and thawing.

**FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS**

Quality Control Testing

Neurite Outgrowth Assay: This lot of ECL was tested at 5 µg/cm² and found to promote neurite outgrowth using PC12 rat pheochromocytoma cells.

IMPORTANT NOTE

Color variations from yellow to dark red may occur in frozen or thawed vials of ECL. These color variations are caused by the interaction of CO₂ with the buffer and phenol red present in the product. Such color variations are normal and do not affect the efficacy of the product.

References:

1. Kleinman, H.K., *et al.*, Biochemistry **25**: 312, 1986.
2. Kleinman, H.K., *et al.*, Biochemistry **21**: 6188, 1982.
3. Hadley, M.A., *et al.*, J. Cell. Biol. **101**: 1511, 1985.
4. Friday, B., *et al.*, J. Cell. Biol. **149**: 657-666, 2001.

Neurite Outgrowth Assay Protocol

1. Coat 6 well dishes with **ECL**, at a concentration of 2.5-10 µg/cm². Incubate 1 hour at 37°C or overnight at 4°C. Aspirate and rinse 2 times with PBS before adding cell suspension.
2. Prepare a cell suspension at 2x10⁴ cells/ml in RPMI-1640/10% horse serum/5% FBS. Add 2 ml of cell suspension to each well.
3. Prepare NGF (Catalog # 01-170) as described on certificate of analysis to a final concentration of 1 µg/ml. Add 20 ng/ml NGF.
4. Incubate plates at 37°C for 3-5 days.
5. Record neurite outgrowth at each ECL concentration.