

---

## Certificate of Analysis

### ECL Cell Attachment Matrix

(entactin-collagen IV-laminin)

Catalog # 08-110

Lot # 0607035935

**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/cm<sup>2</sup>. 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/cm<sup>2</sup> 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 CO<sub>2</sub> 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/cm<sup>2</sup>. 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 2x10<sup>4</sup> 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.