V. 2.0

Laminin: HiLyte Fluor 488™
Source: Engelbreth-Holm-Swarm mouse tumor
Cat. # LMN02

Upon arrival store at 4°C (desiccated)
See datasheet for storage after reconstitution

Background Information

The Extracellular Matrix (ECM) is composed of collagen, non-collagenous glycoproteins and proteoglycans. These components are secreted from cells to create an ECM meshwork that surrounds cells and tissues. The ECM regulates many aspects of cellular function, including the cells dynamic behavior , cytoskeletal organization and intercellular comminucation (1).

Laminin the best known member of a family of basement membrane glycoproteins that play a role in cell adhesion, migration, growth and differentiation (2). Laminins also promote neurite outgrowth and regeneration (3). Many of laminin's functions are mediated by integrin cell surface receptors (2). Laminins are heterotrimers, composed of an α, β and γ subunit (4). Laminin-1 from Engelbreth-Holm-Swarm (EHS) mouse tumor tissue has the composition $\alpha 1\beta 1\gamma 1$ (also termed A1B1B2) and has an approximate molecular weight 850 kD, composed of a 400 kD alpha chain, a 225 kD beta and a 225 kD gamma chain (Figure 1).

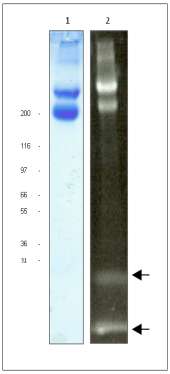
Material

Laminin-1 is purified from EHS tumor tissue and is free of the laminin binding protein entactin which is a common contaminant in some laminin preparations (150 kDa). Protein purity is determined by scanning densitometry of Coomassie Blue stained protein on a 4-20% polyacrylamide gel. The laminin is >90% pure (Figure 1).

The protein is modified to contain covalently linked HiLyte 488™ dyes (5) at random surface lysines. An activated ester of HiLyte 488™ is used to label the protein. Labeling stoichiometry is determined by spectroscopic measurement of protein and dye concentrations. Final labeling stoichiometry is 2-5 dyes per protein molecule (Figure 2). The material is guaranteed to contain <15% of free dye and >85% of dye conjugated to laminin. HiLyte 488™ laminin can be detected using a filter set of 502nm excitation and 527nm emission.

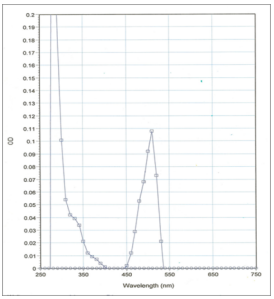
Laminin runs as individual subunits on SDS-PAGE with an apparent molecular weight of 400 and 225 kDa (Figure 1). LMN02 is supplied as an off white lyophilized powder. Each vial of LMN02 contains 20 µg protein.

Figure 1: HiLyte 488™ Laminin Purity Determination



Legend: 20 µg of unlabeled laminin (Lane 1) and 20 µg of HiLyte 488™ laminin (Lane 2) was separated by electrophoresis in a 4-20% SDS-PAGE system. The unlabeled protein was stained with Coomassie Blue and visualized in white light. The labeled protein was visualized under UV light. The alpha subunit runs at 400 kDa (top band) while the beta and gamma subunits run as a 225 kDa doublet (lower band). Arrows indicate unincorporated dye. In this example unincorporated dye = 13%. Protein quantitation was determined with the Precision Red™ Protein Assay Reagent (Cat. # ADV02). Mark12 molecular weight markers are from Invitrogen.

Figure 2: Absorption scan of HiLyte 488™ laminin in solution-



Legend: LMN02 was diluted with Milli-Q water and its absorbance spectrum was scanned between 250 and 750 nm. In this example, HiLyte 488™ labeling stoichiometry was calculated to be 3.5 dyes per laminin protein using the absorbancy maximum for HiLyte 488™ at 527 nm and the Beer-Lambert law. Dye extinction coefficient when protein bound is 70,000$M^{-1}cm^{-1}$.

Storage and Reconstitution

Shipped at ambient temperature. The lyophilized protein can be stored desiccated to <10% humidity at 4°C for 6 months in the dark. For reconstitution, briefly centrifuge to collect the product at the bottom of the tube and resuspend to 1 mg/ml with 20 µl cold distilled water. The protein will then be in the following buffer: 100 mM PIPES pH 7.2, 1% dextran and 5% (w/v) sucrose. Avoid excessive mixing as this can cause protein aggregation. The concentrated protein should be aliquoted into experiment sized amounts, snap frozen in liquid nitrogen and stored at –70°C where it is stable for 6 months. For working concentrations, further dilution of the fluorescent laminin should be made in a suitable buffer or tissue culture media. HiLyte 488™ laminin is a labile protein and should be handled with care. Avoid repeated freeze-thaw cycles.

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Biological Activity Assay

Proteolytic degradation of the ECM is a critical step during cell invasion and is necessary for both physiological and pathological processes. HiLyte 488™ laminin can be used as an ECM substrate to monitor invasion through observation of ECM degradation (6).

Product Uses

- Cell invasion assays (6)
- FACS analysis of laminin binding cells (7)

References

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3. Edgar D. et al. 1984. The heparin-binding domain of laminin is responsible for its effects on neurite outgrowth and neuronal survival. *EMBO J.* **3**: 1463-1468.
4. Burgeson R.E. et al. 1994. A new nomenclature for the laminins. *Matrix Biol.* **14**: 209-211.
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6. Kelly T. et al. 1994. Invadopodia promote proteolysis of a wide variety of extracellular matrix proteins. *J. Cellular Physiol.* **158**: 299-308.
7. Tronchin G. et al. 1997. Expression and identification of a laminin-binding protein in Aspergillus fumigates conidia. *Infection & Immunity* **65**: 9-15.

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