

Biotin Labeled Laminin

Source: Engelbreth-Holm-Swarm mouse tumor

Cat. # LMN03

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 communication (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).

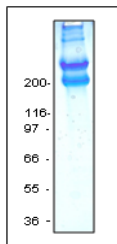
Material

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 2).

The protein is modified to contain covalently linked biotins at random surface lysines. An activated ester of biotin is used to label the protein. The biotin label has been modified to contain a 14 atom spacer between the biotin and the laminin, this improves the accessibility of biotin to avidin/streptavidin. Labeling efficiency is determined by the ability to detect 10ng of biotinylated laminin using alkaline phosphatase labeled streptavidin (Figure 2).

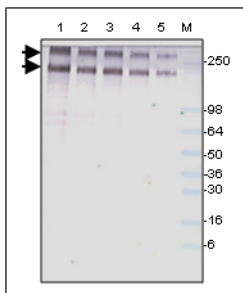
Laminin runs as individual subunits on SDS-PAGE with an apparent molecular weight of 400 and 225 kDa (Figure 1). LMN03 is supplied as an white lyophilized powder. Each vial of LMN03 contains 20 μ g protein.

Figure 1: Biotinylated Laminin Purity Determination



Legend: 20 μ g of unlabeled laminin 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 alpha subunit runs at 400 kDa (top band) while the beta and gamma subunits run as a 225 kDa doublet (lower band). Protein quantitation was determined with the Precision Red™ Protein Assay Reagent (Cat. # ADV02). Mark12 molecular weight markers are from Invitrogen.

Figure 2: Detection of Biotinylated Laminin



Legend: Serial dilutions of biotinylated laminin were separated by electrophoresis on a 4-20% SDS-polyacrylamide gel, blotted to a PVDF membrane, probed with a 1:500 dilution of streptavidin alkaline phosphatase (Sigma) and detected with 1-Step NBY/BCIP reagent™ (Pierce). Lane1, 100ng; Lane 2, 50ng; Lane 3, 40ng; Lane 4, 20ng; Lane 5, 10ng of biotinylated laminin. Lane M, SeeBlue™ molecular weight markers (Invitrogen). Arrows indicate biotinylated laminin subunits (400kD and 225 kD).

Storage and Reconstitution

Shipped at ambient temperature. The lyophilized protein can be stored desiccated to <10% humidity at 4°C for 6 months. For reconstitution, briefly centrifuge to collect the product at the bottom of the tube and resuspend to 1 mg/ml with 20 μ l 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 biotinylated laminin should be made in a suitable buffer or tissue culture media. Biotinylated laminin is a labile protein and should be handled with care. Avoid repeated freeze-thaw cycles.

Biological Activity Assay

Laminins have been shown to promote neurite outgrowth (3).

Product Uses

- Studies requiring laminin binding to solid avidin/streptavidin surfaces(5)

References

1. Guidebook to the extracellular matrix and adhesion proteins. 1993. Oxford University Press. Ed. Kreis T and Vale R.
2. Scheele S et al. 2007. Laminin isoforms in development and disease. *J. Mol. Med.* **85**: 825-836.
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.
5. Lehnert M, et al. 2011. Adsorption and conformation behavior of biotinylated fibronectin on streptavidin-modified TiO (X) surfaces studied by SPR and AFM. *Langmuir*, **27**, 7743-7751.

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