

## Full-length Biolaminin 521 and Biolaminin 221, in combination, increase differentiation of PSC-derived cardiomyocytes to 85 %

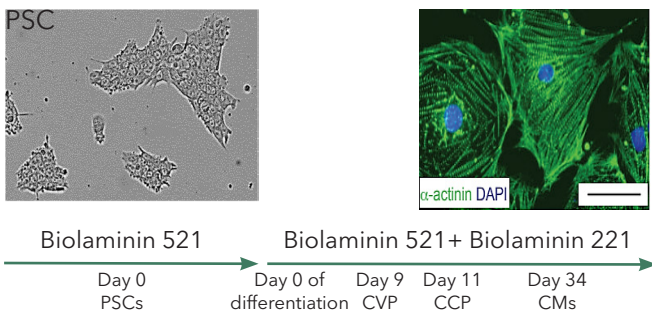
Pluripotent stem cell (PSC)-derived cardiomyocytes play a key role in therapies of the future, serving directly as cellular therapy, as source material for tissue-specific extracellular vesicles, and as a platform for drug development. This requires standardized, reproducible, and efficient derivation of cardiomyocytes.

This application note describes a published method on how to derive xeno-free and defined cardiovascular progenitor (CVPs) cells and cardiomyocytes (CMs) at high efficiency with a combination of Biolaminin 521 and 221 substrates (Yap L. *et al.* 2019 and 2023). The method is twice as efficient as other standardized substrates. The cells mature and display critical functional features, such as contraction and electrophysiological signatures, improving heart function over time after successful integration into infarcted pig heart.

Biolaminin can also be used to functionalize hydrogels, for tissue-specific modelling, and 3D printing, improving CMs maturity and muscle striation.

### FIGURE 1

Combined LN521 + 221 substrates for the generation of cardiovascular progenitor cells (CVPs) and cardiomyocytes (CMs)



Biolaminin 521



Biolaminin 221

### Benefits:

- Efficient generation of PSC-derived cardiomyocytes on Biolaminin 521 + 221, for 2D/3D applications
- Contracting cells already at day 9
- Preclinical evidence of functional integration into the heart

### Product features:

- Full-length human recombinant laminins
- Xeno-free and chemically defined
- From research to clinical applications



**Direct link to Biolaminin 521 LN (LN521) information online**



**Direct link to Biolaminin 221 LN (LN221) information online**

The protocol combines Biolaminin 521 + Biolaminin 221 for efficient, defined and xeno-free differentiation of pluripotent cells into cardiovascular progenitors (CVPs) by day 9, committed cardiac progenitors (CCPs) by day 11 and functional mature cardiomyocytes (CMs) at day 34 [1&2]. Characterization and *in vivo* integration of the generated CVPs and CMs are further described in Figures 2-6.

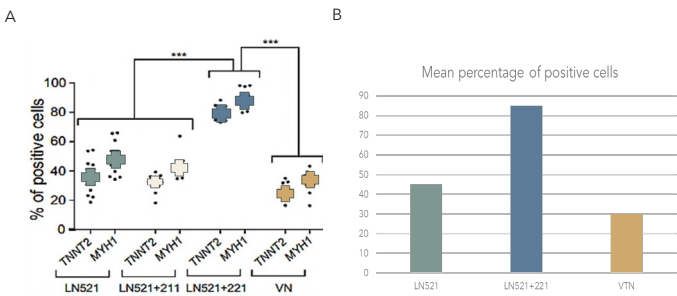
Immunocytochemistry:  $\alpha$ -actin (green) with clear cytoplasmic sarcomeric striations and nucleus stained with DAPI (blue). Scale bar = 100  $\mu$ M.

# Cardiomyocyte differentiation

on a combination of full-length Biolaminin® 521 (LN521) and Biolaminin 221 (LN221) substrates

**FIGURE 2**

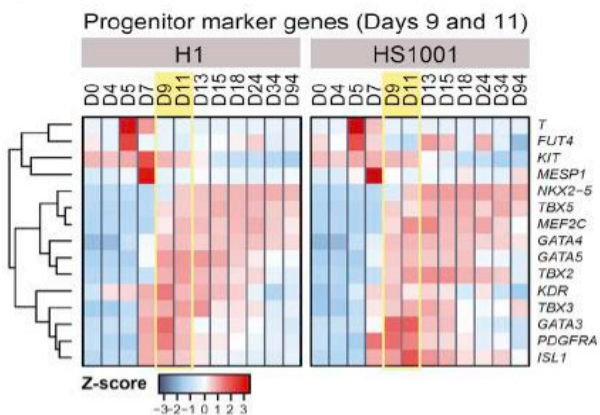
Improved efficiency of CMs differentiation with a combination of Biolaminin 521 and Biolaminin 221



A) Significantly increased number of CMs with a combination of Biolaminin 521 + 221 reaching 85% positive cells (flow cytometry analysis), compared to Biolaminin 521 alone or vitronectin (VN).  
 B) Percentage of CMs based on flow cytometry analysis. [1], figure modified.

**FIGURE 3**

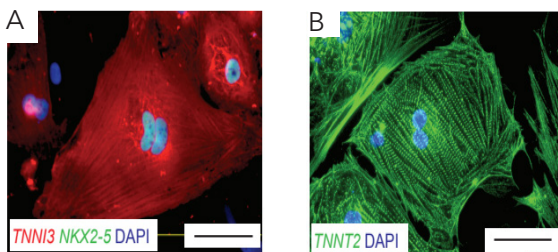
Gene expression during the 94-day differentiation protocol



Characterization of gene expression during the differentiation protocol, from PSCs to mature CMs in two different cell lines, with CVPs emerging on day 9 (D9) and CCPs on day 11 (D11), as highlighted in the figure. [1]

**FIGURE 4**

Characterization of PSC-derived CMs with immunocytochemistry at day 34



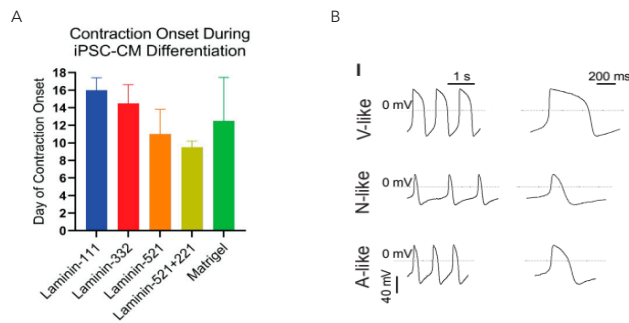
A) A) Immunocytochemistry detecting cardiac-specific Troponin I (TNNI3) (red) and Homeobox protein Nkx-2.5 (NKX2-5) (green). B) Troponin T (TNNT2) (green) showing clear cytoplasmic sarcomeric striations; nuclei stained with DAPI (blue). Scale bar = 100 μM [1].

**REFERENCES**

[1] Yap et al. Cell Reports, 2019. In Vivo Generation of Post-infarct Human Cardiac Muscle by Laminin-Promoted Cardiovascular Progenitors  
 [2] Yap et al. npj regenerative medicine, 2023. Pluripotent stem cell-derived committed cardiac progenitors remuscularize damaged ischemic hearts and cardiomyopathy.  
 [3] Barnes AM et al. Bioengineering (Basel), 2022. Differentiating Human Pluripotent Stem Cells to Cardiomyocytes Using Purified Extracellular Matrix Proteins.  
 [4] Wolfe JT et al. Front Cardiovasc. Med. 2023 3D-bioprinting of patient-derived cardiac tissue models for studying congenital heart disease.

**FIGURE 5**

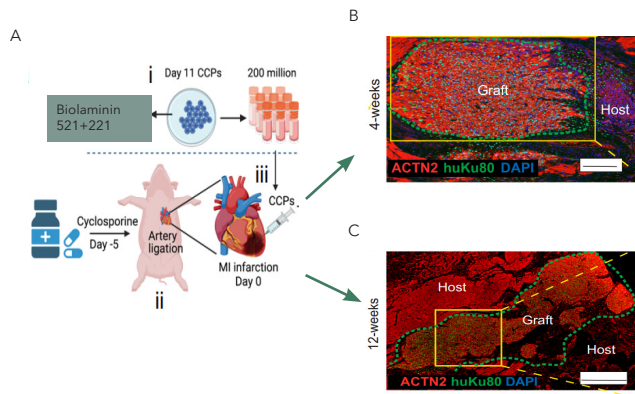
Robust differentiation and functionality



A) Contraction onset during PSC-CM differentiation. The combination of Biolaminin 521 and 221 leads to the fastest differentiation, with contraction starting at day 9.5 ± 0.5. Matrigel, an undefined tissue extract, leads to the highest variation among the substrates [3]. B) Action potentials of ventricular (V)-, nodal (N)- and atrial (A)-like CMs [1].

**FIGURE 6**

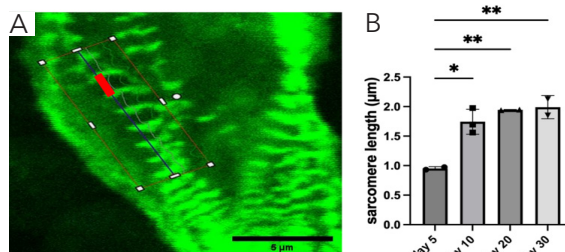
Successful transplantation and integration of cardiac-committed cells



A) Transplantation of LN521+221 derived CCPs into permanent myocardial infarcted heart. B-C) Successful long-term integration of transplanted cells demonstrated by cardiac muscle genes ACTN2 (red) and huKu80 (green) at 4- and 12-weeks post-transplantation (B and C, respectively). Scale bar = 100 μm. [2]

**FIGURE 7**

3D bioprinted PSC-derived myocardial constructs with Biolaminin 521 functionalization



A) Measurement of sarcomere length (red bar). Scalebar = 5 μm. (B) Sarcomere length progressively increased for constructs derived from healthy donors. Sarcomere length was 0.95 μm on day 5, reaching approximately 1.99 μm on day 30, p<0.05.