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# Recombinant Human Laminin-521 Supports PSC Survival in Essential 8™ Medium During Critical Transitions

ThermoFisher SCIENTIFIC

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## ABSTRACT

Feeder-free culture of human pluripotent stem cells (PSCs) in the Essential 8™ Medium system paired with a truncated form of recombinant human Vitronectin (rhVTN-N) supports long term maintenance of normal PSC morphology, pluripotency, and karyotype in the absence of rho-associated coiled-coil kinase (ROCK) inhibitors. However, in certain applications requiring single cell dissociation of PSCs and subsequent manipulation required for gene editing and high throughput screening, inclusion of ROCK inhibitors is required to ensure consistent recovery of PSCs. We tested multiple matrices to identify surfaces that provide maximal support of PSCs cultured in the Essential 8™ Medium system in various applications: somatic cell reprogramming, feeder-dependent to feeder-free transitioning, clonal expansion, and routine PSC culture. Here we show that recombinant human rhLaminin-521 provides the best overall performance, including improved recovery and survival following passage of singularized PSCs in the absence of ROCK inhibitors. In addition, rhLaminin-521 was shown to support long term culture of PSCs, while maintaining normal PSC morphology, pluripotency, karyotype, and trilineage differentiation potential. Several aspects of the process workflow for PSC passaging and culture were evaluated for optimization and versatility with the rhLaminin-521 matrix; these included matrix coating concentration, duration, and temperature, cell seeding density, passaging reagent, and inclusion of RevitaCell™ Supplement, a cocktail containing a pro-survival small molecule coupled with antioxidants and free radical scavengers. For downstream applications in which recovery of PSCs from low density seeding is required, coupling of rhLaminin-521 with RevitaCell™ Supplement provided increased cell recovery from a cell seeding density of 150 cells/cm$^2$. rhLaminin-521 also supported transition of feeder-dependent PSCs to the feeder-free Essential 8™ Medium system better than other matrices tested. Use of rhLaminin-521 increased feeder-free cloning efficiency for neonatal human dermal fibroblasts following somatic cell reprogramming using Cytotune™-iPS 2.0 Sendai Reprogramming Kit. Together, these data demonstrate the robustness of Essential 8™ Medium coupled with rhLaminin-521 in supporting a broad range of PSC applications.

## MATERIALS

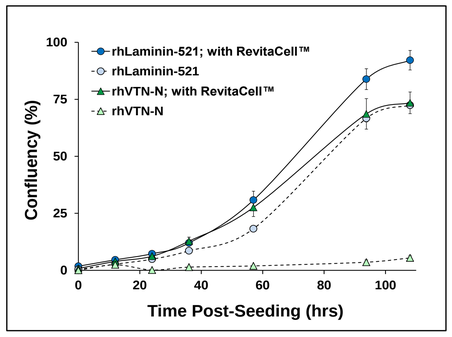
100 µg rhLaminin-521 (Cat. No. A29248)
1 mg rhLaminin-521 (Cat. No. A29249)
Essential 8™ Adaptation Kit (Cat. No. A25935)
Essential 8™ Medium (Cat. No. A15170-01)
RevitaCell™ Supplement (Cat. No. A26445-01)
rhVitronectin-N (Cat. No. A14700)
Geltrex™ (Cat. No. A14133)
iMatrix-511 (Nippi Cat. No. T303)
Gibco® Human Episomal iPSC Line (Cat. No. A18945)
PSC 4-Marker ICC Kit (Cat. No. A24881)
TaqMan® hPSC Scorecard™ Panel (Cat. No. A15870)
CytoTune™-iPS 2.0 Sendai Reprogramming Kit (Cat. No. A16518)
Vector Red Alkaline Phosphatase Substrate Kit (Vector Labs Cat. No. SK-5100)

## INTRODUCTION

Recombinant human Laminin 521 (rhLaminin-521) is a recombinant human protein that provides a xeno-free, defined surface for feeder-free culture of human pluripotent stem cells (PSCs). As an extracellular matrix protein expressed in the inner cell mass of the blastocyst, rhLaminin-521 provides a physiologically relevant environment mimicking the human stem cell niche. Here we show that in combination with Essential 8™ Medium, rhLaminin-521 demonstrates robust and versatile support throughout the PSC workflow.

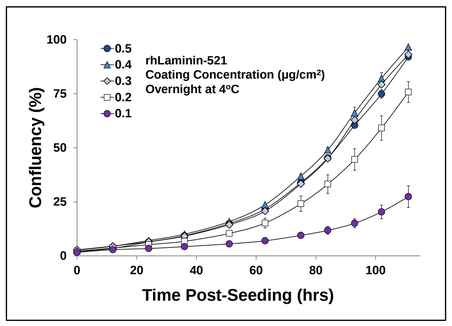
## RESULTS

**Figure 1. rhLaminin-521 provides robust recovery for PSCs passaged as singularized cells without inclusion of ROCK inhibitor**



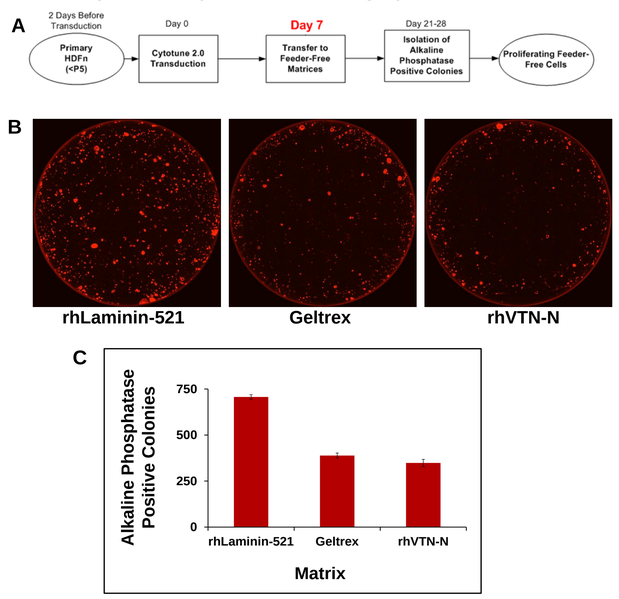

- Gibco® Human Episomal induced Pluripotent Stem Cells (iPSCs) were cultured in Essential 8™ Medium and passaged with TrypLE Select™ at a cell seeding density of 12,500 cells/cm$^2$ onto 0.5 µg/cm$^2$ rhLaminin-521 or rhVTN-N with/without inclusion of RevitaCell™ Supplement. Confluency post-seeding was monitored using the IncuCyte Zoom.
- Using rhLaminin-521, PSCs reached passaging confluency within 3-4 days without requiring addition of RevitaCell™ Supplement (cocktail containing a ROCK inhibitor)

**Figure 2. For routine passaging of PSCs, rhLaminin-521 supports efficient recovery at a range of coating concentrations**



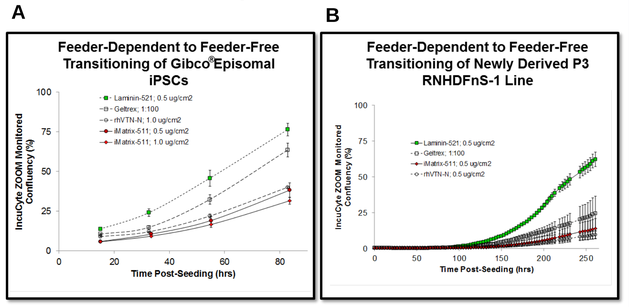

- CMBS2-1 iPSCs (an in house human fibroblast derived line) were cultured in Essential 8™ Medium and passaged with TrypLE Select™ at a cell seeding density of 12,500 cells/cm$^2$ onto plates coated overnight at 4ºC with rhLaminin-521 at the indicated range of concentrations. Confluency was determined using the IncuCyte Zoom.
- rhLaminin-521 coating concentrations as low as 0.2 µg/cm$^2$ supported PSC recovery, with overnight coating at 4ºC. Comparable recovery can also be obtained by using higher cell seeding densities and/or including RevitaCell™ Supplement (data not shown).

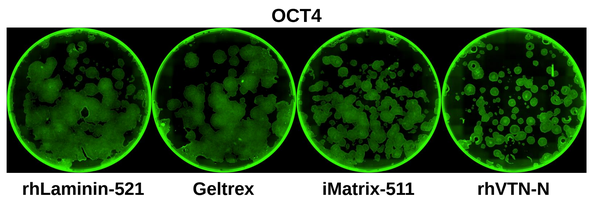
**Figure 3. Use of rhLaminin-521 improves reprogramming efficiency of primary fibroblasts using Cytotune™ 2.0**




- (A) Human neonatal dermal fibroblasts (HDFn) were reprogrammed using the CytoTune™-iPS 2.0 Sendai Reprogramming Kit workflow.
- (B-C) At day 7, transduced HDFns were passaged onto rhLaminin-521, rhVTN-N at 0.5 µg/cm$^2$, or Geltrex at 1:100. At day 20, the number of emerging colonies per well of a 6-well plate was determined by staining for alkaline phosphatase and whole well imaging using the IncuCyte Zoom.
- Incorporating rhLaminin-521 into the CytoTune™-iPS 2.0 workflow demonstrated a significant increase in reprogramming efficiency.

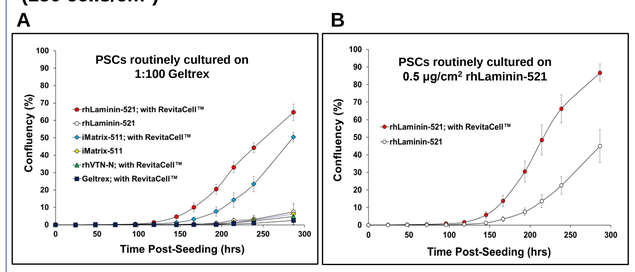
**Figure 4. rhLaminin-521 provides optimal PSC survival during transition from feeder-dependent to feeder-free Essential 8™**



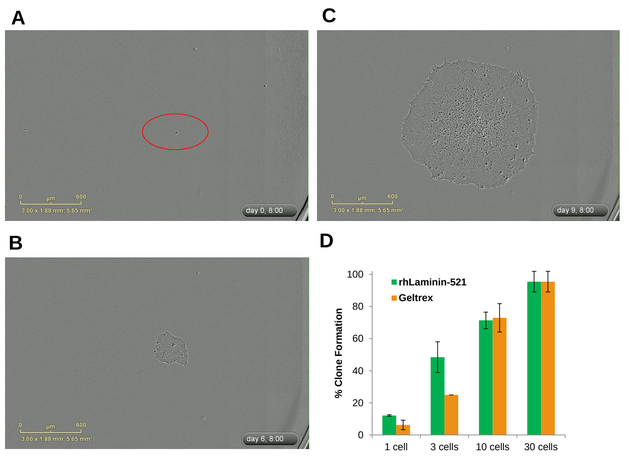

- rhLaminin-521 supported maximal recovery of both an established PSC line (Gibco® Human Episomal iPSCs) (A) and a newly derived iPSC line at passage 3 (B) during transition from feeder-dependent to feeder-free culture in Essential 8™ Medium compared to indicated matrices. The Essential 8™ Adaptation Kit was used for this process. Confluency over the first passage was monitored using the IncuCyte Zoom.
- (C) Pluripotency of Gibco® Human Episomal iPSCs 5 days post feeder-dependent transfer to Essential 8™ Medium was assessed by staining for OCT4. Representative whole well images indicate rhLaminin-521 to promote greatest PSC attachment and recovery during this critical transition.

**Figure 5. rhLaminin-521 coupled with RevitaCell™ Supplement provides increased PSC recovery from clonal seeding densities (150 cells/cm$^2$)**




- (A) Combining Essential 8™ Medium, rhLaminin-521 and RevitaCell™ Supplement provides a robust culture environment for recovery and growth of PSCs seeded at low cell seeding densities. This combination is recommended for applications in which PSCs will undergo increased manipulation and low density seeding.
- (B) Prior to applications requiring low density seeding, adaptation to rhLaminin-521 for 3 passages is recommended for optimal PSC recovery as routine passaging on this matrix increases cell attachment and growth.

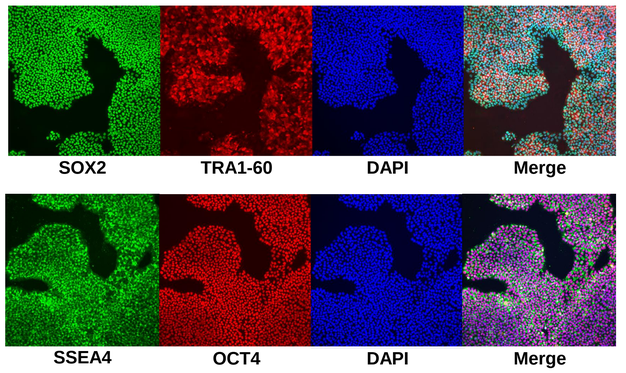
**Figure 6. rhLaminin-521 coupled with Essential 8™ RevitaCell™ Supplement supports improved clonal isolation of PSCs**



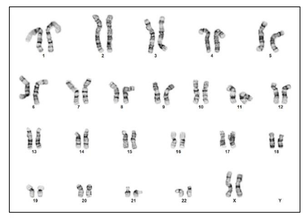

- (A-C) Single viable FACS Sorted TRA1-60 positive cells can form clonal colonies of PSCs when grown with Essential 8™ Medium, rhLaminin-521 and RevitaCell™ Supplement.
- (D) Seeding of 1-3 PSCs into rhLaminin-521-coated 96 well dishes doubles clone formation efficiencies compared to PSCs seeded on Geltrex. Key applications include clonal isolation of CRISPR/Cas9 or TALEN edited PSCs.

**Figure 7. rhLaminin-521 supports maintenance of normal PSC properties**

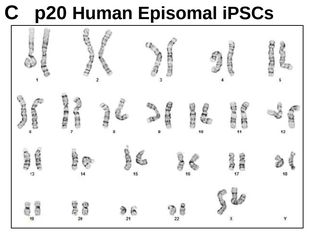
A p20 Human H9 ESCs



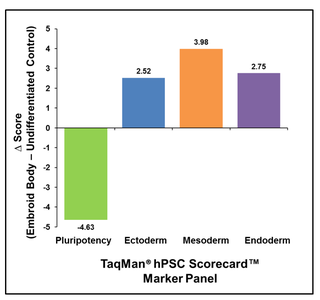

B p20 Human H9 ESCs



C p20 Human Episomal iPSCs



D p17 Human H9 ESCs




- (A) Human H9 ESCs cultured in Essential 8™ Medium on 0.5 µg/cm$^2$ rhLaminin-521 and passaged with TrypLE Select™ maintain normal morphology and expression of pluripotency markers over 20 passages as assessed using the PSC 4-Marker ICC Kit.
- (B-C) Normal karyotype was maintained over 20 passages for 2 PSC lines: H9 ESCs and Gibco® Human Episomal iPSCs.
- (D) PSC trilineage differentiation potential was confirmed using the TaqMan® hPSC Scorecard™ Panel. Embryoid bodies were generated from p17 H9 ESCs and assessed for Scorecard™ differentiation markers in comparison to an undifferentiated control.

## CONCLUSIONS

- When used with Essential 8™ Medium, rhLaminin-521 provides robust recovery for PSCs passaged as singularized cells without inclusion of ROCK inhibitor while maintaining normal morphology, pluripotency, differentiation potential and karyotype.
- rhLaminin-521 promotes cell attachment and survival for success in a broad range of critical applications in the PSC workflow. These include PSC reprogramming, recovery from low density cell seeding, and transition from feeder-dependent to feeder-free culture.
- The **Essential 8™ Adaptation Kit**, (Cat. No. A25935) composed of Essential 8™ Basal Medium, Essential 8™ Supplement, and 100 µg rhLaminin-521, provides a complete system for feeder dependent transition.

## REFERENCES

1. Rodin et al. Nature Protocols. pp2354-2368 (2014).

## ACKNOWLEDGEMENTS

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## TRADEMARKS/LICENSING