

Data Sheet

PluriSTEM®-XF Recombinant Vitronectin

Extracellular Matrix Protein

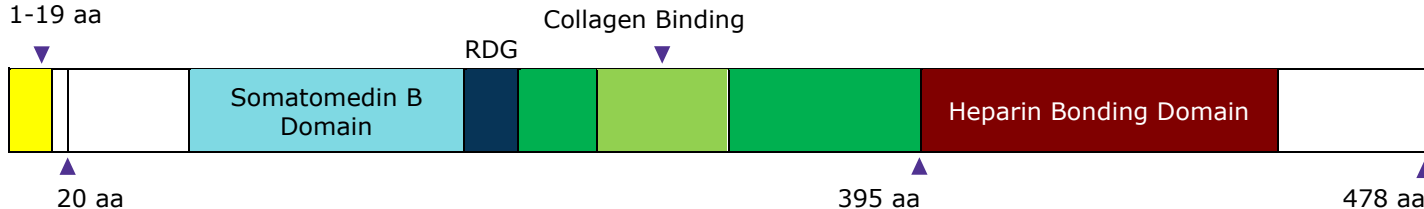
CC130**Pack Size: 500 µg****Store at -20 °C****FOR RESEARCH USE ONLY****Not for use in diagnostic procedures. Not for human or animal consumption.**

Background

PluriSTEM® Recombinant Vitronectin is an effective xeno-free alternative to Matrigel® and has been validated to successfully culture human ES and iPS cells using PluriSTEM® Human ES/iPS Medium (Cat. No. SCM130, SCM132), E8 and NutriStem® human ES cell growth media.

PluriSTEM® Recombinant Vitronectin is a truncated form of human vitronectin (gene 20-398 aa fragment) that was constructed with codon optimization, expressed in *E. coli* and purified as inclusion bodies. The final product was refolded using a unique "temperature shift inclusion body refolding" technology and chromatographically purified. The final product is considered xeno-free.

Signal Domain
1-19 aa



Source

Produced in *E. coli*.

Purity

Product is ≥ 92% pure by SDS-PAGE.

Appearance

Solution is clear and free of particulates.

Presentation

0.5 mg/mL in 20 mM pH 8.0 Tris-HCl buffer with a proprietary formulation of sucrose, EDTA, arginine, and DTT. Product has been 0.2 µm filtered.

Quality Control Testing

Each lot is tested on human ES cells (H1) cultured using 5-10 µg recombinant vitronectin in 1 mL human ESC medium per well (6-well plate).

Storage and Handling

Store at –20 °C until expiration date on vial. Once thawed, product is stable at 2-8 °C for two weeks. Thawed vitronectin can be aliquoted and stored at –20 °C. Avoid additional freeze-thaw cycles.

Protocols

Suggested Coating Protocol for Human ES/iPS

All procedure should be performed under aseptic conditions in a biological safety cabinet.

1. Thaw Recombinant Vitronectin at room temperature.
2. Dilute sufficient amounts of Recombinant Vitronectin to a final concentration of 10 µg/mL with 1X PBS (Cat. No. BSS-1006-B) (see Table 1). Dilutions should be made in a 15 mL polypropylene conical tube. Mix gently. Do not vortex.

Table 1: Volumes recommended for coating cultureware.

Culture Vessel	Vitronectin Solution
6 well	1.5 mL
12 well	0.5 mL
60 mm	2 mL
100 mm	6 mL
T-25	3 mL
T-75	8 mL

3. Transfer the required volume of diluted Recombinant Vitronectin into a culture well. Gently shake the plate to spread the coating solution across the entire well.
4. Incubate at least 1 hour at room temperature or overnight at 4 °C. Coated cultureware can be stored at 2-8 °C for 2-3 days. Before use, allow coated plates to come to room temperature for 30 minutes.
5. Remove the Recombinant Vitronectin coating solution before addition of medium or cells (no extra wash step is necessary). Do not allow the wells to dry out.

Transitioning hES/iPS Cells to Vitronectin coated plates

Due to its enhanced stability, Recombinant Vitronectin may be used for growing human ES and iPS in serum-free, feeder-free medium that are dissociated using either enzymatic or non-enzymatic methods. When E8 medium is used, follow non-enzymatic method for passaging hES colonies. Cells cultured in PluriSTEM® may be dissociated following either enzymatic or non-enzymatic methods. The following protocols are designed for 6-well plates; make adjustment as needed.

Non-Enzymatic Passaging

1. Aspirate the medium from hES/iPS cell culture. Wash each well with 2 mL/well 1X PBS buffer (Cat. No. BSS-1006-B). Aspirate.
2. Add 1 mL/well of Enzyme Free Cell Dissociation Solution (Cat. No. S-004-C) and incubate for 2-3 minutes at room temperature. Monitor the cells every 1-2 minutes and stop treatment accordingly. Do not over-dissociate.
Note: Duration of cell dissociation may be cell line dependent. A 2–3-minute incubation time is specific to H9 cells. Treatment should be stopped when the cells begin to separate, or when holes start to appear in the colonies at higher microscope magnification (10x objective).
3. Gently aspirate the dissociation buffer. Wash with 2 mL/well 1X PBS. Aspirate after the wash and add 2 mL/well of pre-warmed culture medium.
4. Gently detach colonies from the well by gentle pipetting with a 5 mL serological pipette. Avoid creating bubbles. The split ratio may range from 1:10 to 1:50 based upon the cell density. Always adjust the split ratio based on the colony morphology at the current passage and the last split ratio. For example, if the colonies are too dense, increase the split ratio; if colonies are too sparse, decrease the split ratio.
Note: Do not try to scrape off all the colonies as this may lead to breaking colonies to suboptimal small pieces.
5. Make sure the Recombinant Human Vitronectin³⁹⁸ coated plate already contains expansion medium before transferring the cells. Add cells. Total volume = 3 mL. Gently shake the plate back and forth to disperse the cells evenly across the surface of the wells. Place the plate in a 37 °C incubator.
6. Change with fresh medium daily and monitor cell morphology and confluence before the next passage.

Enzymatic Passaging

1. Aspirate the medium from hES/iPS cell culture. Wash each well with 2 mL/well DMEM/F12 (Cat. No. DF-041-B) or 1X PBS buffer (Cat. No. BSS-1006-B).
2. Add 1 mL/well PluriSTEM® Dispase®-II Solution (Cat. No. SCM133) and incubate for 5–7 minutes at room temperature. After incubation, visually inspect the colonies under a microscope. The edges of the colonies may appear slightly rounded up and folded back but the overall colony should still be attached to the plate.
3. Gently aspirate the Dispase®-II solution and gently rinse each well once with DMEM/F12. Aspirate.
4. Add 2 mL PluriSTEM® medium to each well and gently detach the colonies using a cell scraper (Sarstedt Cat. No. 83.1832). Use a 5 mL serological pipette to collect the cell aggregates to a 15 mL conical tube. Minimize pipetting up and down as this may break up the colonies to suboptimal small pieces. The process of transferring the cell aggregates to the 15 mL conical tube should be sufficient to break the colonies to sufficient size.
5. Rinse the wells with an additional 2 mL of PluriSTEM® medium per well to collect any remaining cell aggregates. Add the rinse to the 15 mL conical tube.
6. Centrifuge the 15 mL conical tube containing the cell aggregates at 300 x *g* for 5 minutes at room temperature.
7. Aspirate the supernatant. Resuspend the cell aggregates in an appropriate volume of PluriSTEM® or your hESC culture medium for passaging. Pipette and transfer the cell aggregates with a 5 mL serological pipette, taking care not to break the aggregates into single cell suspensions.

Note: For a confluent culture, a split ratio may range up to 1:20. However as culture techniques and cell lines may vary, it is recommended that the users set up a titration of split ratio ranging from 1:5 to 1:8 to determine the optimal split density.

The split ratio can be increased during passages. Always adjust the ratio based on the colony morphology at the current passage and the last split ratio. If the colonies are too dense, increase the split ratio. If colonies are too sparse, decrease the split ratio.

8. Before transferring cells to Recombinant Human Vitronectin³⁹⁸ coated plates, make sure that each well contains expansion medium. Total volume = 3 mL.
9. Add cells. Gently shake the plate back and forth to disperse the cells evenly across the surface of the wells. Place the plate in a 37 °C incubator.
10. Change with fresh medium daily and monitor cell morphology and confluence before the next passage.

Results

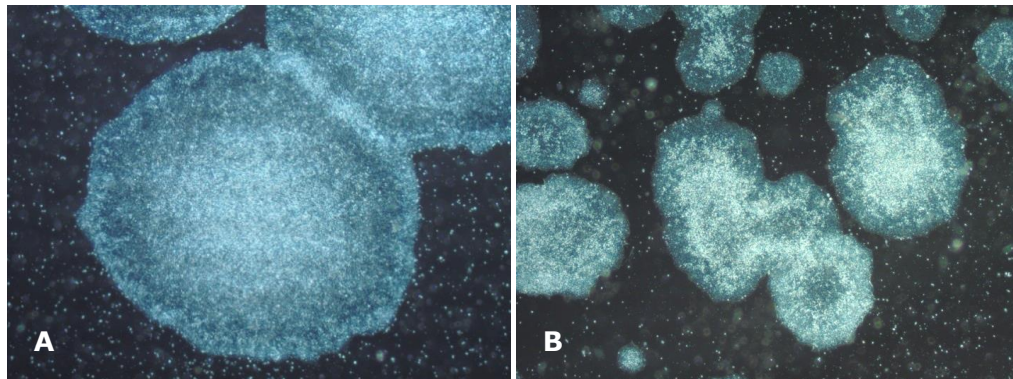


Figure 1: (A) H9 Human ESCs grown on PluriSTEM® Recombinant Vitronectin in PluriSTEM® Human ES/iPS Media and (B) Life Technologies Essential 8 for three passages.

Related Products

Description	Catalogue No.
PluriSTEM® Human ES/iPS Medium	SCM130
PluriSTEM®-XF Human ES/iPS Medium	SCM132
PluriSTEM® Dispase®-II Solution	SCM133
Enzyme Free Cell Dissociation Solution Hank's Based (1X), liquid	S-004-C
PluriSTEM® Freeze Medium	SCM134
ES Cell Characterization Kit	SCR001
Fluorescent Human ES/iPS Cell Characterization Kit	SCR078
Alkaline Phosphatase Detection Kit	SCR004
Human STEMCCA Cre-Excisable Constitutive Polycistronic (OKSM) Lentivirus Reprogramming Kit	SCR545

References

1. Guokai Chen, et al. Chemically defined conditions for human iPSC derivation and culture. Nature Methods. 8, 424-429 (2011).
2. Stefan R. Braam. et al. Recombinant Vitronectin is a Functionally Defined Substrate that Supports Human Embryonic Stem Cell Self-Renewal via αVβ5 integrin. STEM CELLS. Vol 26. Issue 9. 2257-2265 (2008).
3. Hyaman E.G., M.D. Pierschbacher, Y. Ohgren, and E. Ruoslahti. Serum spreading factor (Vitronectin) is present at the cell surface and in tissues. Proc. Natl. Acad. Scie. U.S.A. 80 (13): 4003-4007 (1983).

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