PluriSTEM-XF™ Recombinant Vitronectin

Extracellular Matrix Protein

Cat. # CC130

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES
NOT FOR HUMAN OR ANIMAL CONSUMPTION

pack size: 500µg

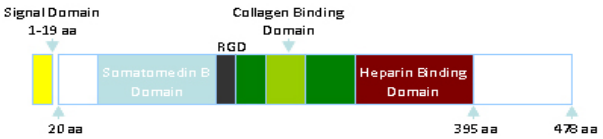
Store at -20ºC

Certificate of Analysis

Background

PluriSTEM™ Recombinant Vitronectin is an effective xeno-free alternative to matrigel™ and has been validated to successully culture human ES and iPS cells using PluriSTEM™ Human ES/iPS Medium (Millipore 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 inclusing 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.



Source

Produced in *E. coli.*

Purity

Product is ≥ 95% pure by SDS-PAGE.

Appearance

Solution is clear and free of particulates.

Presentation

0.5 mg/mL, sterile-filtered, in 20 mM pH 8.0 Tris-HCl buffer, with a proprietary formulation of NaCl, KCl, EDTA, arginine, DTT and glycerol.

Quality Control Assay

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℃ until expiration date on vial. Once thawed, product is stable at 2 - 8℃ for two weeks. Thawed vitronectin can be aliquoted and stored at -20℃. Avoid additi onal freeze-thaw cycles.

Suggested Coating Protocol for Human ES/iPS

Note: 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	1.5ml
12 well	0.5ml
60 mm	2 ml
100 mm	6ml
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℃. Coated cultureware can be stored at 2-8℃ 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.

SPECIES LEGEND: **H** Human **Ca** Canine **M** Mouse **R** Rat **Rb** Rabbit **B** Bovine **P** Porcine **WR** Most Common Vertebrates

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Human ES Cell Culture Protocol using PluriSTEM™ Recombinant Vitronectin

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. 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 (x10 objective).

3. Gently aspirate the dissociation buffer. Wash with 2mL/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 Vitronectin^{398} 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 ℃ 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 (Starstedt 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 from1:5 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 Vitronectin^{398} 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 ℃ incubator.

10. Change with fresh medium daily and monitor cell morphology and confluence before the next passage.

Results

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

Related References

Guokai Chen, et al. Chemically defined conditions for human iPSC derivation and culture. Nature Methods. 8, 424-429 (2011).

Stefan R. Braam. et al. Recombinant Vitronectin is a Functionally Defined Substrate that Supports Human Embryonic Stem Cell Self-Renewal via aVb5 integrin. STEM CELLS. Vol 26. Issue 9. 2257-2265 (2008).

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).

RELATED PRODUCTS

cat #		Description
SCM130		PluriSTEM™ Human ES/iPS Medium
SCM132		PluriSTEM-XF™ Human ES/iPS Medium
SCM133		PluriSTEM Dispase-II Solution
S-004-C		Enzyme Free Cell Dissociation Solution Hank's Based (1X), liquid
SCM134		PluriSTEM™ Freeze Medium
SCR001		ES Cell Characterization Kit
SCR002		ES Cell Marker Sample Kit
SCR078		Fluorescent Human ES/iPS Cell Characterization Kit
FCSC100107		FlowCellect™ Human iPS Cell Characterization Kit
SCR004		Alkaline Phosphatase Detection Kit
SCR545		Human STEMCCA Cre-Excisable Constitutive Polycistronic (OKSM) Lentivirus Reprogramming Kit