

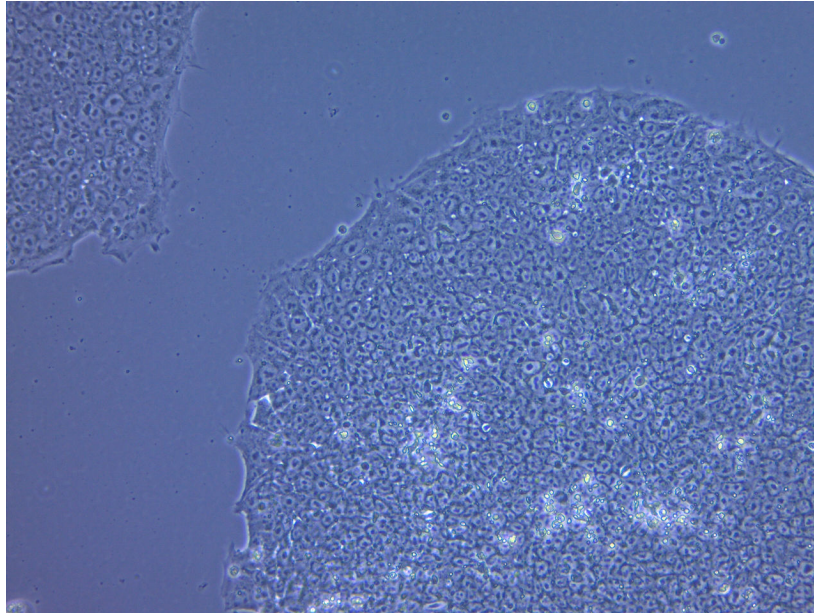
Vitronectin XF™
User Protocol


Fig. 1. Colony of human iPS cells growing on Vitronectin XF™ coated non-TC treated 6-well plate

Vitronectin XF™ (Product No. S2153-500UG)

- 500 µg Vitronectin XF™, sterile filtered, 250 µg/ml
 Stability and storage: 6 months at -80 °C or 4 weeks at 4 °C, undiluted

Vitronectin XF™ Evaluation Kit (Product No. S2159)

Qty.	Component
• 500 ug	Vitronectin XF™
• 100 ml	Dilution Buffer (store at RT)
• 1 ml	Ca ⁺⁺ /Mg ⁺⁺ Concentrate (500X, store at -80°C)
• 100 ml	Cell Release Buffer (store at RT)
• 8 ea	6-well Polystyrene Suspension Plates, Sterile Pre-wrapped

Vitronectin XF™ is a xeno-free single-component cell attachment factor intended to support growth and differentiation of human pluripotent stem cells (hPSCs) under serum-free, feeder-free conditions. Primorigen's Vitronectin XF™ is a recombinant fusion protein that contains the entire human Vitronectin sequence (NCBI Reference Seq. NP_000629.3), and is to be used for coating of sterile, non-tissue culture treated polystyrene dishes and plates. Coating should be performed on the day of use although plates with coating solution may be incubated overnight at 37°C in a humidified CO₂ incubator.

Important!

- Coat only **NON**-tissue culture treated polystyrene plasticware
- Use polypropylene tubes for performing all dilutions

Primorigen Biosciences • 510 Charmany Drive • Madison, WI 53719

☎ 608.441.8332 • ✉ info@primorigen.com • www.primorigen.com

Trademark and Patent Information: Primorigen®, and Primorigen Biosciences® are registered trademarks of Primorigen Biosciences. Vitronectin XF™ is a trademark of Primorigen Biosciences. All other trademarks are property of their respective holders.

Protocols for use of Vitronectin XF™

Materials required for coating

- Non-tissue culture treated polystyrene plasticware (supplied 6-well plates or alternative)
- Sterile polypropylene conical tubes (50 mL suggested)
- Sterile transfer pipettes
- Dilution Buffer (add 200 μ L of 500X $\text{Ca}^{++}/\text{Mg}^{++}$ Concentrate to the bottle of Dilution Buffer)

Materials for establishment and passaging of cells

- Glass 5 ml pipets (Fisher 13-678-27E)
- Prewarmed serum-free culture medium
- Cell Release Buffer (acceptable results may also be obtained with Lonza Versene #17-711E)

Coating plasticware with Vitronectin XF™

*Note: This protocol should be performed under aseptic conditions in a Biological Safety Cabinet. **USE ONLY NON-TISSUE CULTURE TREATED PLASTICWARE.***

1. Thaw the vial of Vitronectin XF™ at room temperature.
2. Dilute sufficient amount of Vitronectin XF™ to 10 μ g/ml with room temperature Dilution Buffer to cover the desired plasticware (see Table 1 on page 5). Dilutions should be made in a 50 ml polypropylene conical tube.
The required dilution is 1:25, e.g. 240 μ l Vitronectin XF™ diluted into 5.76 ml Dilution Buffer is sufficient for one 6-well plate. Optimal concentration of Vitronectin XF™ may vary depending upon cell type- try 15 and 20 μ g/ml if attachment seems weak. Store remaining undiluted Vitronectin XF™ at 4 degrees
3. Mix gently (do not vortex).
4. Deposit required volume of diluted Vitronectin XF™ in a pool in the center of the well (e.g. 1 ml/well in a 6-well plate). After adding substrate into the desired wells, gently shake the plate horizontally, side to side and forward-backward to spread the coating solution across the entire well or plate surface.
5. Incubate 3 hr to overnight at 37 °C in a humidified CO₂ incubator.
6. Before use, remove Vitronectin XF™ solution and quickly rinse once with 1 ml/well (or an equal volume) of Dilution Buffer before addition of medium or cells. Do not allow wells to dehydrate.
7. Medium may be added and used immediately or the plate can be held at 37 °C in a CO₂ incubator until addition of the cells.

Primorigen Biosciences • 510 Charmany Drive • Madison, WI 53719

☎ 608.441.8332 • ✉ info@primorigen.com • www.primorigen.com

Trademark and Patent Information: Primorigen®, and Primorigen Biosciences® are registered trademarks of Primorigen Biosciences. Vitronectin XF™ is a trademark of Primorigen Biosciences. All other trademarks are property of their respective holders.

Converting cells from Matrigel™ to growth on Vitronectin XF™

It is essential to prevent proteolysis of cell surface proteins when preparing colonies of pluripotent cells for establishment on Vitronectin XF™ coated plates. The following non-enzymatic steps should be used for colonies growing on Matrigel™ or other feeder-free ECM substrates. Suggested volumes are for 6-well plates; adjust as needed.

1. Aspirate the medium from hPSC culture, rinse with 1 ml/well Cell Release Buffer and aspirate the well.
2. Add 1 ml/well Cell Release Buffer.
3. Leave at room temperature for **9 minutes**.
4. Gently aspirate Cell Release Buffer.
5. Gently detach cell colonies from each well with gentle pipetting of 2 ml/well growth medium, using a 5 glass ml pipette. Take care to minimize the breakup of colony clumps, ensuring that single cells are not generated.
6. Dropwise evenly distribute the colony suspension into each well of a prepared Vitronectin XF™ coated 6-well plate at desired split ratio (1:3 to 1:6 split recommended, depending on specific cell line).
7. Ensure that each well has at minimum 2 ml/well medium; add additional medium if needed.
8. Move the plate in several quick, short, back-and-forth and side-to-side motions to disperse cells across the surface of the wells. Place the plate in a 37 °C incubator.
9. Refeed colonies daily and monitor morphology and confluency. Passage when adjacent colonies begin to touch.

Passaging stem cells grown on Vitronectin XF™

The split ratios of 1:3 to 1:6 from Vitronectin XF™ to Vitronectin XF™ have resulted in desirable attachment and confluence rates. It is recommended to use these ratios depending on the confluence of the starting wells. Suggested volumes are for 6-well plate, adjust as needed.

1. Aspirate the medium from hPSC culture, rinse with 1 ml/well Cell Release Buffer and aspirate the well.
2. Add 1 ml/well Cell Release Buffer.
3. Leave at room temperature for **3-4 minutes**.
4. Gently aspirate Cell Release Buffer.
5. Gently detach cell colonies from each well with gentle pipetting with 2 ml/well growth medium using a 5 glass ml pipette. Take care to minimize the breakup of colony clumps, ensuring that single cells are not generated. Some minimal scraping may be required.

Primorigen Biosciences • 510 Charmany Drive • Madison, WI 53719

☎ 608.441.8332 • ✉ info@primorigen.com • www.primorigen.com

Trademark and Patent Information: Primorigen®, and Primorigen Biosciences® are registered trademarks of Primorigen Biosciences. Vitronectin XF™ is a trademark of Primorigen Biosciences. All other trademarks are property of their respective holders.

6. Dropwise evenly distribute the colony suspension into each well of a prepared Vitronectin XF™ coated 6-well plate at desired split ratio (1:3 to 1:6 split recommended, depending on specific cell line).
7. Ensure that each well has at minimum 2 ml/well medium; add additional medium if needed.
8. Move the plate in several quick, short, back-and-forth and side-to-side motions to disperse cells across the surface of the wells. Place the plate in a 37 °C incubator.
9. Refeed colonies daily and monitor morphology and confluency. Passage when adjacent colonies begin to touch.

Table 1. Recommended volumes for coating

	Wells/dish				Dish/flask size			
	96	24	12	6	60 mm	100 mm	T25	T75
area per well (cm ²)	0.3	2	3.8	9.6	28.3	78.5	25	75
recommended volume per well (ml)	0.05	0.25	0.50	1.0	3.0	6.0	3.0	5.0
minimum volume per well (ml) at 78 µl/cm ²	0.02	0.16	0.30	0.75	2.20	6.12	1.95	5.85

Available separately

Vitronectin XF™	S2153-500UG
Cell Release Buffer (100 ml)	S2072-100ML
Dilution Buffer (100 ml)	S2161-100ML
6-well Polystyrene Plates (100/cs)	S2118-CS

Primorigen Biosciences • 510 Charmany Drive • Madison, WI 53719

 ☎ 608.441.8332 • ✉ info@primorigen.com • www.primorigen.com

Trademark and Patent Information: Primorigen®, and Primorigen Biosciences® are registered trademarks of Primorigen Biosciences. Vitronectin XF™ is a trademark of Primorigen Biosciences. All other trademarks are property of their respective holders.

LIMITED USE LICENSE
“For Research Use Only”

This product is to be used for Research Purposes only and for no other purpose. The term “Research Purposes” means scientific research programs directly under the user’s control, which are specifically directed to the purposes of internal research and not for any Commercial Purpose. The purchase and/or use of this product conveys to the user the non-transferable right to use the product and the components of the product in research conducted by the user. The user will not reformulate the Product and shall not sell or otherwise transfer this product, its components, or materials made therefrom to any third party. User shall not use this product or its components for Commercial Purposes until it has obtained a license for this purpose. The term “Commercial Purposes” shall mean any activity by a party for consideration and may include, but is not limited to, use of the product or its components (i) in manufacturing, (ii) to provide a service, information or data to a third party (e.g. fee for sample testing), (iii) for therapeutic, diagnostic or prophylactic purposes, (iv) for resale, whether or not such product or its components are resold for use in research, or (v) in a quality control or quality assurance process for the manufacture of a product for sale. The use of this product by the user constitutes agreement with the terms of this limited use label license for Primorigen Biosciences Inc.”

Primorigen Biosciences Inc. warrants that its products will conform to the standards stated in its product specification sheets in effect at the time of shipment when used as directed. Primorigen Biosciences Inc. will replace, free of charge, any product that does not conform to the specifications, but only when used as directed. This warranty limits the liability of Primorigen Biosciences Inc. to only the replacement of the product. **THIS WARRANTY IS EXCLUSIVE, AND PRIMORIGEN BIOSCIENCES INC. MAKES NO OTHER WARRANTY, EXPRESS OR IMPLIED, INCLUDING WITHOUT LIMITATION, ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE.** The stated express warranties, and the remedy provided for breach thereof, are in lieu of all other liability or obligations of PRIMORIGEN BIOSCIENCES INC. for any damages whatsoever arising out of or in connection with the delivery, use, performance, or the inability to use any of its products. **IN NO EVENT SHALL PRIMORIGEN BIOSCIENCES INC. BE LIABLE UNDER ANY LEGAL THEORY (INCLUDING BUT NOT LIMITED TO CONTRACT, NEGLIGENCE, STRICT LIABILITY IN TORT, OR WARRANTY OF ANY KIND) FOR ANY INDIRECT, SPECIAL, INCIDENTAL, CONSEQUENTIAL, OR EXEMPLARY DAMAGES (INCLUDING BUT NOT LIMITED TO LOST PROFITS).** Without limiting the effect of the preceding sentence, the maximum liability, if any, of Primorigen Biosciences Inc., shall not exceed the purchase price paid by PURCHASER for the product. The use of the product by the user constitutes user's understanding of and agreement with the terms of this limited warranty and limited liability policy.

Primorigen Biosciences • 510 Charmany Drive • Madison, WI 53719

☎ 608.441.8332 • ✉ info@primorigen.com • www.primorigen.com

Trademark and Patent Information: Primorigen®, and Primorigen Biosciences® are registered trademarks of Primorigen Biosciences. Vitronectin XF™ is a trademark of Primorigen Biosciences. All other trademarks are property of their respective holders.