PRIMORIGEN BIOSCIENCES®

Vitronectin XF™ User Protocol

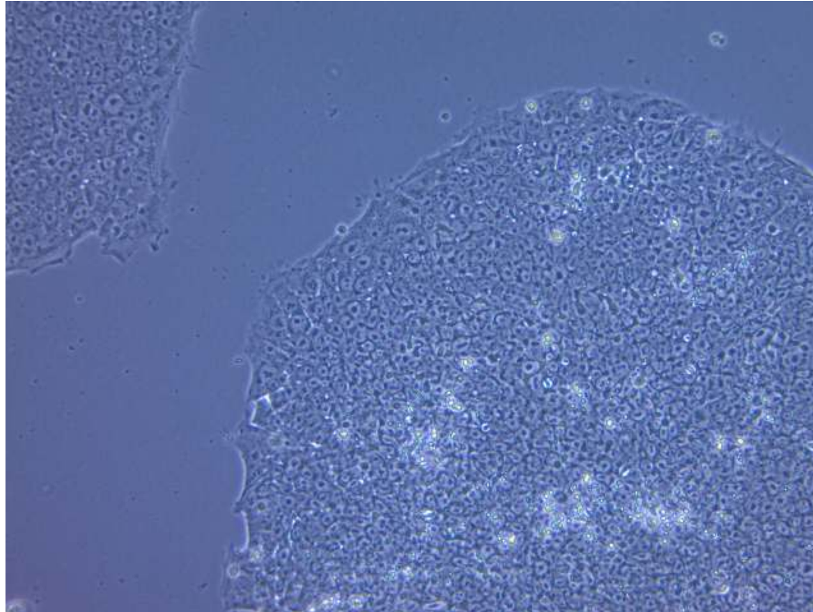

Fig. 1. Colony of human iPS cells growing on Vitronectin XF™ coated non-TC treated 6-well plate

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

- 500 µg Vitronectin XF™, sterile filtered, 250 µg/ml
  Stability and storage: 1 year at -20 to -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,)
• 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 CO_2 incubator.

Important!

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

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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 uL of 500X Ca^{++}/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 µg/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 µl 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 µg/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 CO_2 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 CO_2 incubator until addition of the cells.

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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 desireable 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.

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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 $\mu l/cm^2$	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

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