Formerly

Product	Vitronectin XF™
Primorigen Product Number	S2153-500UG
Nucleus Biologics Part Number	10230
Vial Contents	2 mL, 500 µg protein at 250 µg/mL
Storage Temperature	-80 °C

INSTRUCTIONS FOR USE

Components Needed:

- Dilution Buffer: Phosphate Buffered Saline (PBS) 1X with 0.5 mM calcium chloride or CellAdhere™ Dilution Buffer (Stem Cell Technologies #07183)
- Vitronectin XF™ (Part #10230)
- Non-tissue culture treated polystyrene plasticware
- Cell Dissociation Buffer (CRB) such as Versene (Lonza #17-711E)

Coating plasticware with Vitronectin XF™

Should be performed under aseptic conditions in a Biological Safety Cabinet.

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

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 above). 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 °C
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 minumun of 1 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.

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 CRB and aspirate the well.
2. Add 1 mL/well CRB.
3. Leave at room temperature for **9 minutes**.
4. Gently aspirate CRB.
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 CRB and aspirate the well.
2. Add 1 mL/well CRB.
3. Leave at room temperature for **3-4 minutes**.
4. Gently aspirate CRB.
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.
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, 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.