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Cellular Dynamics international

Vitronectin, truncated recombinant human (rhVTN-N)

Cat. No.:	Size:	Store at –80°C
A15068SA	1 mL (0.5 mg/mL)	

Part no. A15069PISSA **Pub. no.** MAN0007163 **Rev.** 22 August 2012

Description

The truncated recombinant human vitronectin (rhVTN-N), corresponding to the amino acid fragment 62–478 of human vitronectin expressed in *E. coli*, is purified from inclusion bodies and refolded for use as a substrate for the feeder-free culture of human pluripotent stem cells (PSCs) in Essential 8™ medium.

Working Concentration

The optimal working concentration of vitronectin is cell line dependent and must be determined empirically. We recommended using a final coating concentration of 0.1–1.0 µg/cm^2 on the culture surface, depending on your cell line. We routinely use vitronectin at 0.5 µg/cm^2 for human PSC culture.

Prior to coating culture vessels, calculate the working concentration of vitronectin using the formula below and dilute the stock appropriately. Refer to Table 1 for culture surface area and volume required.

$$\text{Working Conc.} = \text{Coating Conc.} \times \frac{\text{Culture Surface Area}}{\text{Volume Required for Surface Area}}$$

$$\text{Dilution Factor} = \frac{\text{Stock Concentration (0.5 mg/mL)}}{\text{Working Concentration}}$$

Example for Working Concentration

To coat a 6-well plate at a coating concentration of 0.5 µg/cm^2, you will need to prepare 6 mL of diluted vitronectin solution (10 cm^2/well surface area and 1 mL of diluted vitronectin/well; see Table 1) at the following working concentration:

$$\text{Working conc.} = 0.5\ \mu g/cm^2 \times \frac{10\ cm^2}{1\ mL} = 5\ \mu g/mL$$

$$\text{Dilution factor} = \frac{0.5\ mg/mL}{5\ \mu g/mL} = 100X \text{ (i.e., 1:100 dilution)}$$

Coating Culture Vessels with Vitronectin

Instructions for coating a 6-well culture plate at a coating concentration of 0.5 µg/cm^2 are provided below. For volumes used in other culture vessels, refer to Table 1. To calculate the working concentration of vitronectin used with other coating concentrations and to determine the appropriate dilution factor, use the equations on the previous page.

1. Upon receipt, thaw the vial of vitronectin at room temperature and prepare 60-µL aliquots of vitronectin in polypropylene tubes. Freeze the aliquots at –80°C or use immediately.
2. To coat the wells of a 6-well plate, remove a 60-µL aliquot of vitronectin from –80°C storage and thaw at room temperature. You will need one 60-µL aliquot per 6-well plate.
3. Add 60 µL of thawed vitronectin into a 15-mL conical tube containing 6 mL of sterile DPBS without Calcium and Magnesium (Cat. no. 14190-144) at room temperature. Gently resuspend by pipetting the vitronectin dilution up and down.

   Note: This results in a working concentration of 5 µg/mL (i.e., a 1:100 dilution).
4. Add 1 mL of the diluted vitronectin solution to each well of a 6-well plate (refer to Table 1 for the recommended volumes for other culture vessels). When used to coat a 6-well plate (10 cm^2/well) at 1 mL/well, the final concentration will be 0.5 µg/cm^2.

5. Incubate the coated plates at room temperature for 1 hour.

   Note: The culture vessel can now be used or stored at 2–8°C wrapped in laboratory film for up to a week. Do not allow the vessel to dry. Prior to use, pre-warm the culture vessel to room temperature for at least 1 hour.

6. Aspirate the vitronectin solution and discard. It is not necessary to rinse off the culture vessel after the removal of vitronectin. Cells can be passaged directly onto the vitronectin-coated culture vessels.

Cells cultured in Essential 8™ Medium (Cat. no. A15045SA) on vitronectin-coated culture vessels should be passaged using 0.5 mM EDTA in DPBS. Use of enzymes such as collagenase and dispase for passaging these cells results in compromised viability and attachment.

Table 1 Volume of diluted vitronectin required

Culture Vessel	Approx. Surface Area	Volume of Diluted Vitronectin
6-well plate	10 cm^2 per well	1.0 mL per well
12-well plate	4 cm^2 per well	0.4 mL per well
24-well plate	2 cm^2 per well	0.2 mL per well
35-mm dish	10 cm^2	1.0 mL
60-mm dish	20 cm^2	2.0 mL
100-mm dish	60 cm^2	6.0 mL
T-25 flask	25 cm^2	2.5 mL
T-75 flask	75 cm^2	7.5 mL

Technical Support

For additional product and technical information, such as Safety Data Sheets (SDS), Certificates of Analysis, etc., visit our website at **www.lifetechnologies.com/gibco**.
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