Vitronectin, truncated recombinant human (VTN-N)

Description

The truncated recombinant human vitronectin (VTN-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. When used with Essential 8™ Medium, VTN-N has been proven to maintain pluripotency and normal growth characteristics in multiple PSC lines.

Product	Catalog No.	Amount	Storage	Shelf Life*
Vitronectin, truncated recombinant human (VTN-N)	A14700	1 mL (0.5 mg/mL)	Store at –80°C	24 months

* Shelf Life duration is determined from Date of Manufacture, which can be found on the Certificate of Analysis (CoA).

Product Use

For Research Use Only. Not for use in diagnostic procedures.

Important Information

Cells cultured in Essential 8™ Medium (Cat. no. A1517001) on vitronectin-coated culture vessels should be passaged using 0.5 mM EDTA prepared in Dulbecco's Phosphate-Buffered Saline (DPBS) without calcium or magnesium (Cat. no. 14190). Alternatively, cell passaging can be performed using Versene solution (Cat. no. 15040), which is 0.48 mM EDTA in PBS. Use of enzymes such as collagenase and dispase, for passaging these cells results in compromised viability and attachment.

Safety Information

Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Culture Conditions

Culture Type: Adherent feeder-free

Substrate: Vitronectin (VTN-N)

Recommended Media: Complete Essential 8™ medium

Temperature Range: 36°C to 38°C

Incubator Atmosphere: Humidified atmosphere of 5% CO_2. Ensure that proper gas exchange is achieved in culture vessels.

Working Concentration

The optimal working concentration of vitronectin is cell line dependent and must be determined empirically. We recommend using a final coating concentration of 0.5 µg/cm^2 on the culture surface. 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.

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

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

For example, 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:

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

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

Coat Culture Vessels with Vitronectin

Instructions for coating a 6-well culture plate with vitronectin 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 above.

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) 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.

Table 1 Reagent Volumes (in mL per well or per dish)

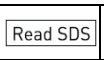
Culture vessel	Approx. surface area	Volume of diluted Vitronectin solution
6-well plate	10 cm^2 per well	1.0 mL per well
12-well plate	4 cm^2 per well	0.4 mL per well
24-well plate	2 cm^2 per well	0.2 mL per well
35-mm dish	10 cm^2	1.0 mL
60-mm dish	20 cm^2	2.0 mL
100-mm dish	60 cm^2	6.0 mL
T-25 flask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

Related Products

Product	Cat. no.
Essential 8™ Medium	A1517001
Dulbecco's PBS (DPBS) without Calcium and Magnesium	14190
UltraPure™ 0.5 M EDTA, pH 8.0	15575
Versene Solution	15040

Explanation of Symbols and Warnings

The symbols present on the product label are explained below:

					
Use by	Temperature limitation	Batch code	Catalog number	Read safety data sheet	Manufacturer

Limited Product Warranty

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For additional technical information such as Safety Data Sheets (SDS), Certificates of Analysis, visit **www.lifetechnologies.com/support**.
For further assistance, email **techsupport@lifetech.com**

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