

## **VTN Coating Protein Product Manual**

## I. Product Introduction

VTN Coating Protein (Vitronectin) is a chemically defined, xenogeneic-free cell matrix that supports the growth and differentiation of human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs). It can be used in combination with ncEpic or ncTarget pluripotent stem cell culture media (or mTeSR, E8).

## II. Product Information

**Table 1: VTN Coat Protein Product Description** 

Product Information	Cat.No.	Amount	Concentration	Storage
Vitronectin	RP01002	1 mg (2 mL)	500 μg/mL	-20°C or -80°C
Truncated VTN-NC				
Purity ≥ 95%				
Endotoxin content ≤ 25 EU/mg				

## III. Instructions for Use

The recommended coating concentration for VTN is 1 μg/cm². For example, a 6-well plate with a growth area of 10 cm<sup>2</sup> per well requires 10 µg of VTN.

Table 2: Recommended Amounts of VTN Working Solution (10 μg/mL) for Different Culture Vessels

Container	Area	Amount of VTN coat protein	
6-Well Plate	10 cm² /well	10 µg	
60-mm Culture Dish	20 cm <sup>2</sup>	20 μg	
100-mm Culture Dish	60 cm <sup>2</sup>	60 μg	
T-25 Culture Flask	25 cm <sup>2</sup>	25 μg	

According to Table 2, a 6-well plate has a total area of 60 cm<sup>2</sup>, and 60 μg of VTN coating protein is required for coating, which equals 120 μL (500 μg/ml). The VTN coating protein can be aliquoted into 120 μL (60 μg) per tube and stored at -20°C or -80°C. When needed, take one tube of VTN coating protein (120 μL, 60 μg), dilute it with DMEM/F12 culture medium to make the working solution, and use it to coat one 6-well plate.

IV. Coating Culture Plates (Using a 6-Well Plate as an Example. The Same Procedure Applies to Other Culture Vessels.)

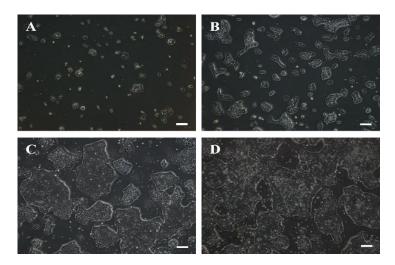
- 1. Thaw one vial of VTN Coating Protein (120  $\mu$ L, 60  $\mu$ g) at room temperature (15–25°C).
- Prepare a 15 mL centrifuge tube. Add 9 mL of DMEM/F12 medium and then transfer the thawed VTN Coating Protein into the medium. Gently mix to dilute. Do not vortex.
- 3. Use the diluted VTN Coating Protein solution immediately for coating. Apply 1.5 mL per well for a 6-well plate.
- 4. Gently tilt the plate to ensure the solution fully covers the bottom surface of each well.



5. Incubate at room temperature (15–25°C) for at least 1 hour before use.

Tips: If not used immediately, seal the plate to prevent evaporation. Store coated plates at 4°C and use within 1 week. Before use, bring the plate to room temperature (15–25°C) for 10–30 minutes.

6. Before seeding cells, tilt the plate and aspirate the coating solution using a pipette tip. Ensure the coated surface is intact without scratches. Do not rinse or add additional solutions.



Morphology of Continuously Cultured hiPSCs in hPSC Complete Medium (NcEpic) on Vitronectin-Coated Plates

Panels A, B, C, and D show the morphology of hiPSCs on Days 1, 2, 3, and 4 of culture, respectively.

Scale bar: 200 µm.