



**LD Biopharma, Inc.**  
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## - PRODUCT DATA SHEET -

**Name of Product:** Recombinant Human Vitronectin (62 - 398aa) Protein  
**Catalog Number:** hRP-0323  
**Manufacturer:** LD Biopharma, Inc.

### Introduction

Human vitronectin (478aa) is a liver derived major plasma glycoprotein that exhibits multiple activities and functions as a cell adhesion molecule and regulator of coagulation. It contains the amino acid structural motif, Arg-Gly-Asp (RGD), which is involved in cell attachment. Human Vitronectin has a mass of 75kDa and circulates as a single-chain moiety of 75kDa and a two-chain moiety of 65kDa and 10kDa. Vitronectin belongs to the group of structurally and functionally homologous adhesive proteins (fibrinogen, fibronectin, Von Willebrand factor), which interact with platelets and the vessel wall in the early stages of blood clotting. When coated on surfaces, very low concentrations of Vitronectin promote endothelial cell attachment and induce spreading and migration of cells in a time- and concentration-dependent fashion. Recent data from James Thomson's laboratory indicated that deletion of C terminal of vitronectin domain displays its capacity as matrix coating protein for both ES and iPS cell cultures when combined with E8 medium<sup>(1)</sup>.

N-terminal human Vitronectin gene (62 - 398 aa) was constructed with codon optimization and expressed in non-fusion protein form in E.coli as inclusion bodies. The final product was refolded using our unique "temperature shift inclusion body refolding" technology and chromatographically purified. Coating this recombinant protein E8, ES or NutriStem medium at concentration of 0.5 ug – 1 ug per cm<sup>2</sup> will benefit for cultures of either human ES cell or iPS cells.

**Gene Symbol:** VTN (62 – 398aa)  
**Accession Number:** NP\_000629  
**Species:** Human  
**Size:** 100 µg  
**Composition:** 0.5 mg/ml, sterile-filtered, in 20 mM pH 8.0 Tris-HCl Buffer, with proprietary formulation of NaCl, KCl, EDTA, arginine, DTT and Glycerol.  
**Storage:** In liquid. Keep at -80°C for long term storage. Product is stable at 4 °C for at least 30 days.



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## Key References

Guokai Chen, et al. *Chemically defined conditions for human iPSC derivation and culture*. Nature Methods. 8, 424-429 (2011)

Stefan R. Braam. Et al. *Recombinant Vitronectin is a Functionally Defined Substrate That Supports Human Embryonic Stem Cell Self-Renewal via  $\alpha$ V $\beta$ 5 integrin*. STEM CELLS. Vol 26. Issue 9. 2257-2265 (2008)

## Application

When coated at 0.5 -1  $\mu\text{g per cm}^2$  and combined with either E8 or Nutristem medium, this recombinant protein can be used as matrix protein for maintaining human ES or iPS in culture.

## Quality Control

1. Purity: > 95% by SDS-PAGE.
2. Biological Activity: When coated onto tissue culture plastics, Human Vitronectin promotes one half maximal attachment of BALB/3T3 cells in serum-free medium at < 0.1  $\mu\text{g / cm}^2$ . Maximum attachment should occur at approximately 0.2  $\mu\text{g / cm}^2$ .

Each Lot was tested using 10 $\mu\text{g/well}$  (in 6 well plate) in Nutristem medium for human ES cells (H9).

## Coating Protocol

1. Dilute the stock solution to the desired concentration with PBS to obtain the final coating solution. To coat plastic surfaces, add 100 $\mu\text{l}$  coating solution at various concentrations of different attachment factors to replicate sets of a 96-well tissue culture plate, or add 1 -1.5 ml to a 6 well plate. Use 1  $\mu\text{g / cm}^2$  of recombinant human Vitronectin.
2. Coat substrates with enough final coating solution to completely cover the surface. Incubate for 1 hour at room temperature or 4 $^{\circ}\text{C}$  overnight.
3. Remove the coating solution and the plates are ready for culturing ES cells. Use 1ml PBS per well, add 5 - 10 $\mu\text{g}$  protein to each well and incubate at 4 $^{\circ}\text{C}$  overnight. After coating, remove PBS solution, the plate is ready for ES cell cultivation.



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## **Recombinant Protein Sequence**

MTRGDVFTMPED EYTVYDDGEEKNNATVHEQVGGPSLTSDLQAQSKGNPEQTPVLKPEEEAPAP  
EVGASKPEGIDSRPETLHPGRPQPPAEELCSGKPFDAFTDLKNGSLFAFRGQYCYELDEKAVR  
PGYPKLIRDVWGI EGPIDAAFTRINCQKTYL FKG SQYWR FEDGVLD PDYPRN ISDGF DGI PDN  
VDAALALPAHSYSGRERVYFFK GKQYWEYQFQH QPSQE ECEGSSLSAVFEHFAMMQRDSWEDIF  
ELLFWGR TSAGTRQPQFISRDWHGVPGQVDAAMAGRIYISGMAPRPSLAKKQRFRRHRNRKGYRS  
QRGHSRGRNQNSRRPSR