



LD Biopharma, Inc.
9924 Mesa Rim Road Suite B
San Diego, CA 92121
Tel: 858-876-8266
<http://www.ldbiopharma.com>

- PRODUCT DATA SHEET -

Name of Product: Recombinant Human VTB Protein
Catalog Number: hRP-0335
Manufacturer: LD Biopharma, Inc.

Introduction

Human VTB (Human vitronectin-Thymosin b4 fusion) is constructed by fusion Vitronectin (62-398 domain) with full length Thymosin b4 at its C-terminal. Human Vitronectin is a liver derived major plasma glycoprotein that exhibits multiple activities and functions as a cell adhesion molecule and regulator of coagulation. Human Thymosins are small polypeptides originally isolated from the thymus, but most are actually present in many other tissues. The archetypical b-Thymosin is Thymosin-b4, which is a cellular constituent in many tissues. In addition to its role as a major actin-sequestering molecule, Thymosin-b4 has a role in tissue repair. Such effects have been shown in animal models of tissue damage and now in human clinical trials. Recently, Recombinant Vitronectin NC terminal domain (62-398aa) has been developed as coating matrix protein for sustaining human ES or iPS cell culture^(1 & 2) and Thymosin-b4 was shown to stimulate regeneration of adult cardiac progenitor cells in a paper published in Nature⁽³⁾.

To develop a unique coating matrix protein for sustaining ES cell culture and benefiting ES to cardiac progenitor differentiation, recombinant human vitronectin (62–398aa) domain and full-length human Thymosin-b4 fusion protein cDNA were expressed in *E. coli* as inclusion bodies, refolded using our unique “temperature shift inclusion body refolding” technology and chromatographically purified. Coating this recombinant VTB protein in proper ES cell or cardiomyocytes culture medium⁽¹⁾ at concentration 0.5ug – 1ug per 1 cm² may benefit for culture of human ES cell and differentiation cardiac progenitor differentiation in vitro.

Gene Symbol: VTB
Accession Number: NP_000629 + NP_066932
Species: Human
Size: 100 µg / Vial



LD Biopharma, Inc.
9924 Mesa Rim Road Suite B
San Diego, CA 92121
Tel: 858-876-8266
<http://www.ldbiopharma.com>

Composition: 1.0 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 -20°C for long term storage. Product is stable at 4 °C for at least 7 days.

Key References

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

Braam, S.R. *et al. Recombinant vitronectin is a functionally defined substrate that supports human embryonic stem cell self-renewal via alphavbeta5 integrin*. Stem Cells 26, 2257–2265 (2008)

Nicola Smart, et al. *Thymosin b4 induces adult epicardial progenitor mobilization and neovascularization*. Vol 445 doi:10.1038/nature05383 (2007)

Applications

1. When coated at 0.5-1 ug/ ml per cm² and combined with cardiomyocytes culture medium, this recombinant protein can be used as matrix protein for benefiting cardiomyocytes differentiation in vitro

Quality Control

1. Purity: > 95% by SDS-PAGE.
2. Endotoxin: < 30 EU per 1 µg of the protein by the LAL method.
3. Biological Activity: When coated onto tissue culture plastic, VTB fusion protein promotes one half maximal attachment of human cardiomytes cells in serum-free medium at < 0.1 µg / cm². Maximum attachment should occur at approximately 0.5 µg / cm².

Coating Protocol

1. Dilute the stock solution to the desired concentration with DPBS to make the final coating solution. To coat plastic surfaces, add 100µl per well of the various concentrations of



LD Biopharma, Inc.
9924 Mesa Rim Road Suite B
San Diego, CA 92121
Tel: 858-876-8266
<http://www.ldbiopharma.com>

attachment factor to replicate sets of a 96-well tissue culture plate, or 2ml per well for a 6 well plate. Use 1µg / cm² of recombinant VTB fusion protein.

Coat substrates with enough final coating solution to completely cover the surface. Incubate for 2 hours at room temperature or overnight at 4°C.

2. Remove the coating solution and immediately rinse the wells with DPBS.
3. Remove the DPBS solution before adding cells.

Recombinant Protein Sequence

MTRGDVFTMPED EYTVYDDGEEKNNATVHEQVGGPSLTSDLQAQSKGNPEQTPVLKPEEEAPAP
EVGASKPEGIDSRPETLHPGRPQPPAEELCSGKPFDAFTDLKNGSLFAFRGQYCYELDEKAVR
PGYPKLIRDVWGIEGPIDAAFTRINCQGKTYLFGKSQYWRFEEDGVLDPDYPRNISDGFDPGIPDN
VDAALALPAHSYSGRERVYFFKKGQYWEYQFQHQP SQE ECEGSSLSAVFEHFAMMQRDSWEDIF
ELLFWGRTSAGTRQPQFISRDWHGVPGQVDAAMAGRIYISGMAPRPSLAKKQRFRRHRNRKGYRS
QRGHSRGRNQNSRRPSRGGGGSGGGGSNIEFSDKPDMAEIEKFDKSKLKKTTETQEKNPLPSKET
IEQEKQAGES