

Product Overview

ACRO offers two GMP-grade DLL4 proteins, <u>GMP-DL4H27</u> (Flagship Product) and GMP-DL4H28 (Complementary Product), designed for iPSC cell culture and differentiation applications. While both proteins undergo similar manufacturing processes with minor procedural variations, they are strategically positioned to address distinct user needs.

Primary Recommendation: <u>GMP-DL4H27</u> (Flagship Product)

As the cornerstone of our DLL4 protein portfolio, GMP Human DLL4 Protein, Fc Tag, Flagship (Cat. No. <u>GMP-DL4H27</u>) is always the optimal choice for new users and standardized workflows. This protein delivers superior activity and consistent performance and is validated across diverse differentiation protocols, making it the first choice for initial testing and implementation.

Complementary Option: GMP-DL4H28

For some specialized applications requiring nuanced optimization, GMP Human DLL4 Protein, Fc Tag (Cat. No. GMP-DL4H28) may demonstrate marginally enhanced effects in specific experimental contexts. We recommend evaluating this product:

- 1. If <u>GMP-DL4H27</u> does not meet performance expectations in your system.
- 2. During process development, parallel screening of both variants could identify the optimal fit for your workflow.

Features

- Designed under ISO 9001:2015 and ISO 13485:2016
- Manufactured and QC tested under a GMP compliance factory
- FDA DMF filed
- Animal-Free materials
- Beta-lactam materials free
- Batch-to-batch consistency
- Stringent quality control tests

Source

GMP Human DLL4 Protein, Fc Tag(GMP-DL4H28) is expressed from human 293 cells (HEK293). It contains AA Ser 27 - Pro 524 (Accession # NP 061947.1).

Predicted N-terminus: Ser 27

Molecular Characterization

DLL4(Ser 27 - Pro 524) Fc(Pro 100 - Lys 330) NP 061947.1 P01857

This protein carries a human IgG1 Fc tag at the C-terminus.

The protein has a calculated MW of 80.7 kDa. The protein migrates as 90 kDa±3 kDa under reducing (R) condition (SDS-PAGE) due to glycosylation.

Endotoxin

Less than 10 EU/mg by the LAL method / rFC method.

Protein A

<5 ppm of protein tested by ELISA.

Host Cell Protein



Sterility

The sterility testing was performed by membrane filtration method described in USP<71> and Ph. Eur. 2.6.1.

Mycoplasma

Negative.

Purity

>95% as determined by SDS-PAGE.

Formulation

Lyophilized from 0.22 µm filtered solution in PBS, pH7.4 with protectants.

Contact us for customized product form or formulation.

Shipping

This product is supplied and shipped with blue ice, please inquire the shipping cost.

Storage

Upon receipt, store it immediately at -20°C or lower for long term storage.

Please avoid repeated freeze-thaw cycles.

This product is stable after storage at:

- -20°C to -70°C for 5 years in lyophilized state;
- -70°C for 12 months under sterile conditions after reconstitution.

GMP Human DLL4 Protein, Fc Tag

Catalog # GMP-DL4H28

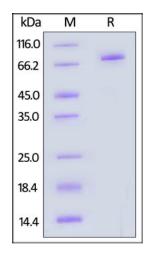


 $< 0.5 \text{ ng/}\mu\text{g}$ of protein tested by ELISA.

Host Cell DNA

<0.02 ng/µg of protein tested by qPCR.

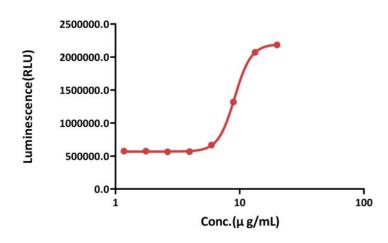
SDS-PAGE



GMP Human DLL4 Protein, Fc Tag on SDS-PAGE under reducing (R) condition. The gel was stained with Coomassie Blue. The purity of the protein is greater than 95%.

Bioactivity-CELL BASE

GMP Human DLL4 Protein, Fc Tag stimulates Human Notch1 (Luc) HeLa Reporter Cell

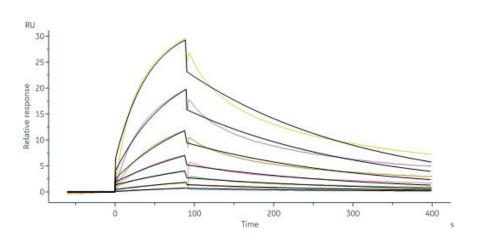


GMP Human DLL4 Protein, Fc Tag (Cat. No. GMP-DL4H28) stimulates Human Notch1 (Luc) HeLa Reporter Cell. The EC50 of the effect is 9.10 μ g/mL (Routinely tested).

Bioactivity-SPR

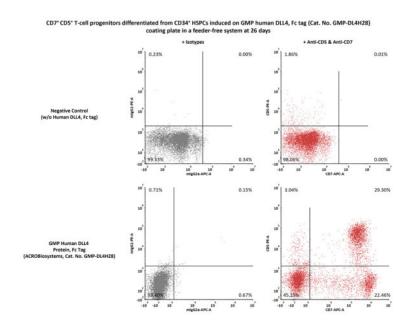






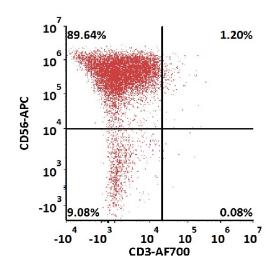
GMP Human DLL4 Protein, Fc Tag (Cat. No. GMP-DL4H28) captured on Protein A Chip can bind Human NOTCH1 Protein, His Tag, premium grade (Cat. No. NO1-H52H3) with an affinity constant between 1.00 nM - 150 nM as determined in a SPR assay (Biacore 8K) (QC tested).

Application Data



CD34+ CD45+ hematopoietic cells were seeded on GMP Human DLL4 Protein, Fc Tag (Cat. No. GMP-DL4H28) - coated plates and differentiated for 26 days, then flow cytometry was used to detect the expression of T-cell progenitor markers, CD7 and CD5. GMP Human DLL4 Protein, Fc Tag (Cat. No. GMP-DL4H28) together with other growth factors could induce the formation of CD7+ and CD7+ CD5+ T-cell progenitors. However, the cells cultured without GMP Human DLL4 Protein, Fc Tag (Cat. No. GMP-DL4H28) - coated plates expressed neither CD5 nor CD7.

Hematopoietic Stem cells differentiate to NK Cells (CD3-CD56+) after 30 days of Culture

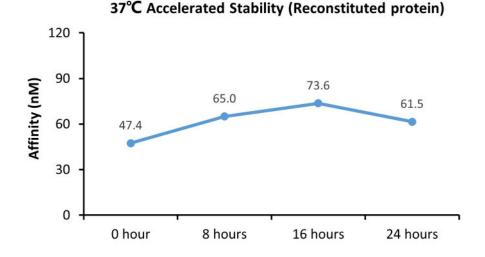


The Recombinant Fibronectin fragment, premium grade (Cat. No. FIN-H5116) combined with GMP Human DLL4 Protein, Fc Tag (Cat. No. GMP-DL4H28), GMP Human VCAM-1 Protein, Fc Tag (Cat. No. GMP-VC1H25) coating on the plate could efficiently induce hematopoietic stem cells differentiation to NK cells, with high expression of CD56+ CD3-.

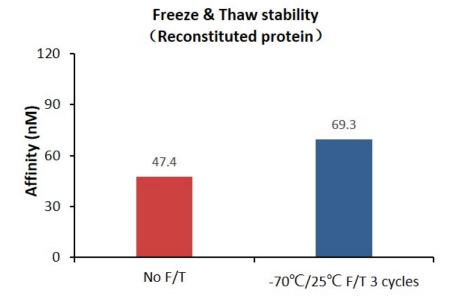




Bioactivity-Stability

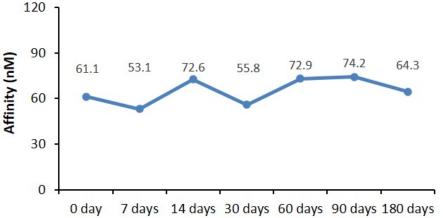


The SPR based assay shows that GMP Human DLL4 Protein, Fc Tag (Cat. No. GMP-DL4H28) is stable at 37°C for 24 hours.



The SPR based assay shows that GMP Human DLL4 Protein, Fc Tag (Cat. No. GMP-DL4H28) is stable after freezing and thawing 3 times.

4°C Accelerated Stability (Reconstituted protein)



The SPR based assay shows that GMP Human DLL4 Protein, Fc Tag (Cat. No. GMP-DL4H28) is stable at 4°C for 180 days.

MANUFACTURING SPECIFICATIONS

ACROBiosystems GMP grade products are produced under a quality management system and in compliance with relevant guidelines: Ph. Eur General Chapter 5.2.12 Raw materials of biological origin for the production of cell-based and gene therapy medicinal products; USP<92>Growth Factors and Cytokines Used in Cell Therapy Manufacturing; USP<1043>Ancillary Materials for Cell, Gene, and Tissue-Engineered Products; ISO/TS 20399-1:2018, Biotechnology - Ancillary Materials Present During the Production of Cellular Therapeutic Products.

ACROBiosystems Quality Management System Contents:

Designed under ISO 9001:2015 and ISO 13485:2016, Manufactured and QC tested under a GMP compliance factory

Animal-Free materials

Materials purchased from the approved suppliers by QA

ISO 5 clean rooms and automatic filling equipment

Qualified personnel

Quality-related documents review and approve by QA



GMP Human DLL4 Protein, Fc Tag





Fully batch production and control records

Equipment maintenance and calibration

Validation of analytical procedures

Stability studies conducted

Comprehensive regulatory support files

Request For Regulatory Support Files (RSF)

ACROBiosystems provide rigorous quality control tests (fully validated equipment, processes and test methods) on our GMP grade products to ensure that they meet stringent standards in terms of purity, safety, activity and inter-batch stability, and each bulk QC lot mainly contains the following specific information:

SDS-PAGE

Protein content

Endotoxin level

Residual Host Cell DNA content

Residual Host Cell Protein content

Biological activity analysis

Microbial testing

Mycoplasma testing

In vitro virus assay

Residual moisture

Batch-to-batch consistency

Background

Delta-like protein 4 (DLL4) is also known as Drosophila Delta homolog 4 (Delta4), which contains one DSL domain and eight EGF-like domains. DLL4 is expressed in vascular endothelium. DLL4 is involved in the Notch signaling pathway as Notch ligand, which can activates NOTCH1 and NOTCH4. DLL4 is involved in angiogenesis and negatively regulates endothelial cell proliferation and migration and angiogenic sprouting. DLL4 can bind to Notch-1 and Notch-4.

