AdipoGen®
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PRODUCT DATA SHEET

AG-40A-0077 29-Oct-2010

DLL4 (human):Fc (human) (rec.)

[Delta-like Protein 4; Delta4]

AG-40A-0077-C010	10 µg
AG-40A-0077-C050	50 µg

Source/Host	HEK 293 cells
MW	~100kDa (SDS-PAGE)
Sequence	Signal peptide and extracellular domain of human DLL4 (aa 1-529) are fused at the C-terminus to the Fc portion of human IgG1.

Handling / Storage

Shipping	BLUE ICE
Short Term Storage	+4°C
Long Term Storage	-20°C

After opening, prepare aliquots and store at -20°C. Avoid freeze/thaw cycles. For maximum product recovery after thawing, centrifuge the vial before opening the cap.

Use / Stability
Working aliquots are stable for up to 3 months when stored at -20°C.

MSDS available at www.adipogen.com or upon request.

Product Specifications

Specificity	Interacts with human Notch1 (as confirmed by flow cytometry).
Biological Activity	Inhibits adipogenesis of 3T3L-1 cells and mesenchymal stem cells (MSCs). Induces Hes-1 in 3T3L-1 cells.
Purity	≥90% (SDS-PAGE)
Formulation	Liquid. 0.2µm-filtered solution in PBS.
Concentration	~0.5mg/ml
Endotoxin Content	<0.1EU/µg purified protein (LAL test; Lonza).

Other Product Data

UniProt link Q9NR61: DLL4 (human) [Precursor]

Adipogen AG
Schützenstrasse 12
4410 Liestal
Switzerland
TEL +41-61-926-60-40
FAX +41-61-926-60-49
info@adipogen.com

Adipogen Corp.
11588 Sorrento Valley Road, Suite 16
San Diego CA 92121-1336
USA
TEL (858) 457-8383
FAX (858) 457-8484
info-us@adipogen.com

Adipogen, Inc.
Room 401, Venture Building B, Songdo TechnoPark,
7-50 Sondo-dong, Yeonsu-gu, Incheon, 406-840,
South Korea
TEL 032-858-1470
FAX 032-831-1470
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Product Description

The Notch ligand delta-like protein 4 (DLL4) is expressed highly and selectively within the arterial endothelium and has been shown to function as a ligand for Notch1 and Notch4. It is induced by VEGF as a negative feedback regulator and acts to prevent overexuberant angiogenic sprouting, promoting the timely formation of a well differentiated vascular network. DLL4-Notch1 signaling regulates the formation of appropriate numbers of tip cells to control vessel sprouting and branching in the mouse retina.

Product Specific References

1. Jagged2 acts as a Delta-like Notch ligand during early hematopoietic cell fate decisions: I. Van de Walle, et al.; Blood **117,** 4449 (2011)

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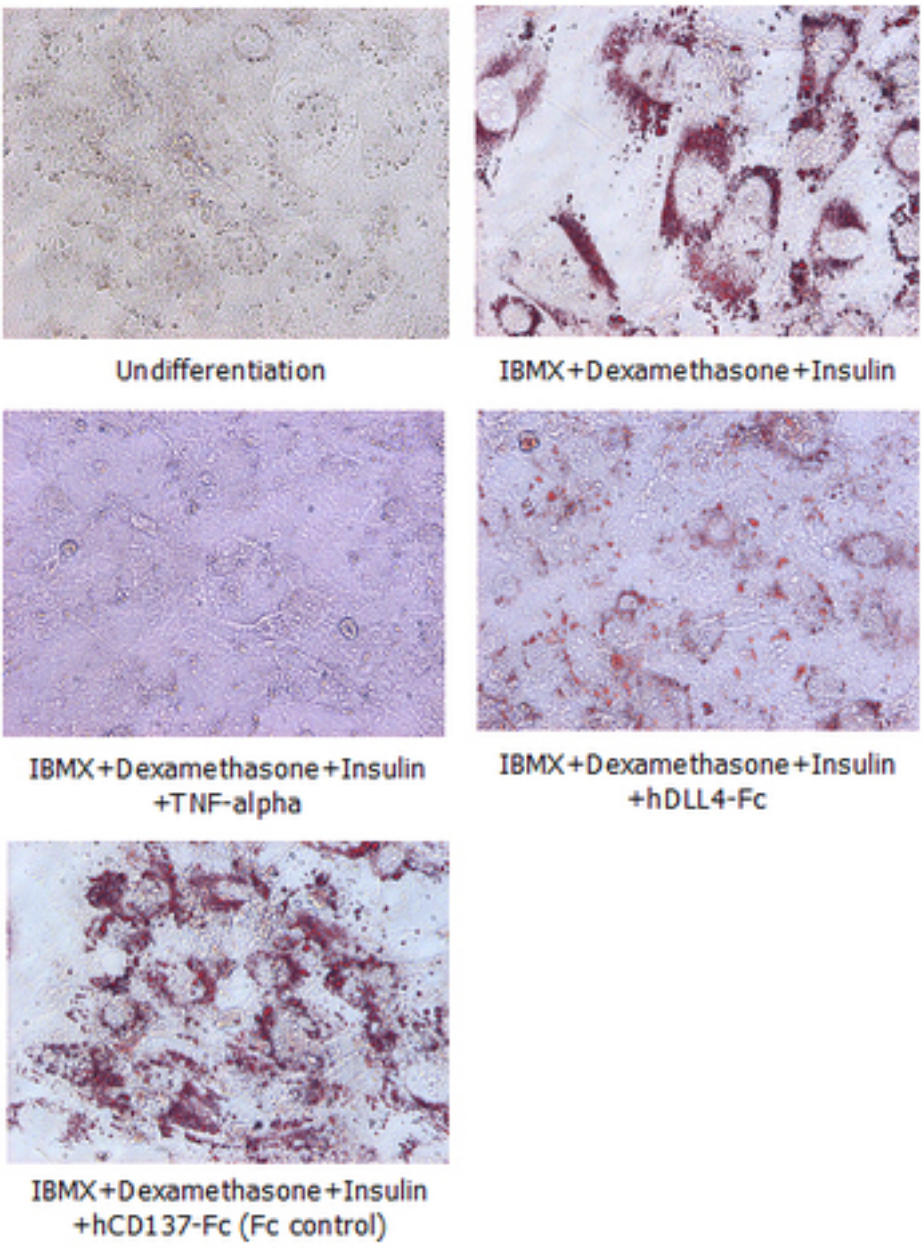

Figure 1: Adipogenesis inhibition of 3T3L-1 cells.
3T3L-1 Cells(mouse pre-adipocyte cells) were maintained in DMEM, supplemented with 10% fetal bovine serum and penicillin-streptomycin. For differentiation of 3T3L-1 Cells, 3T3L-1 Cells were cultured in adipogenic medium which was growth medium supplemented with 1μM Dexamethasone, 0.5mM IBMX, 10μg/ml Insulin (day 0). Medium was changed every 2 days. Staining with Oil Red O was typically performed on day 7. Cells were washed twice with PBS, fixed with 3.7% formalin, and stained with 0.5% filtered Oil Red O in propylene glycol. For negative controls, mouse TNF-α (20ng/ml) was added. Recombinant human DLL4-Fc (5μg/ml) dissolved in DPBS was added to the differentiation medium. These plates were then used to differentiate 3T3L-1 Cells.

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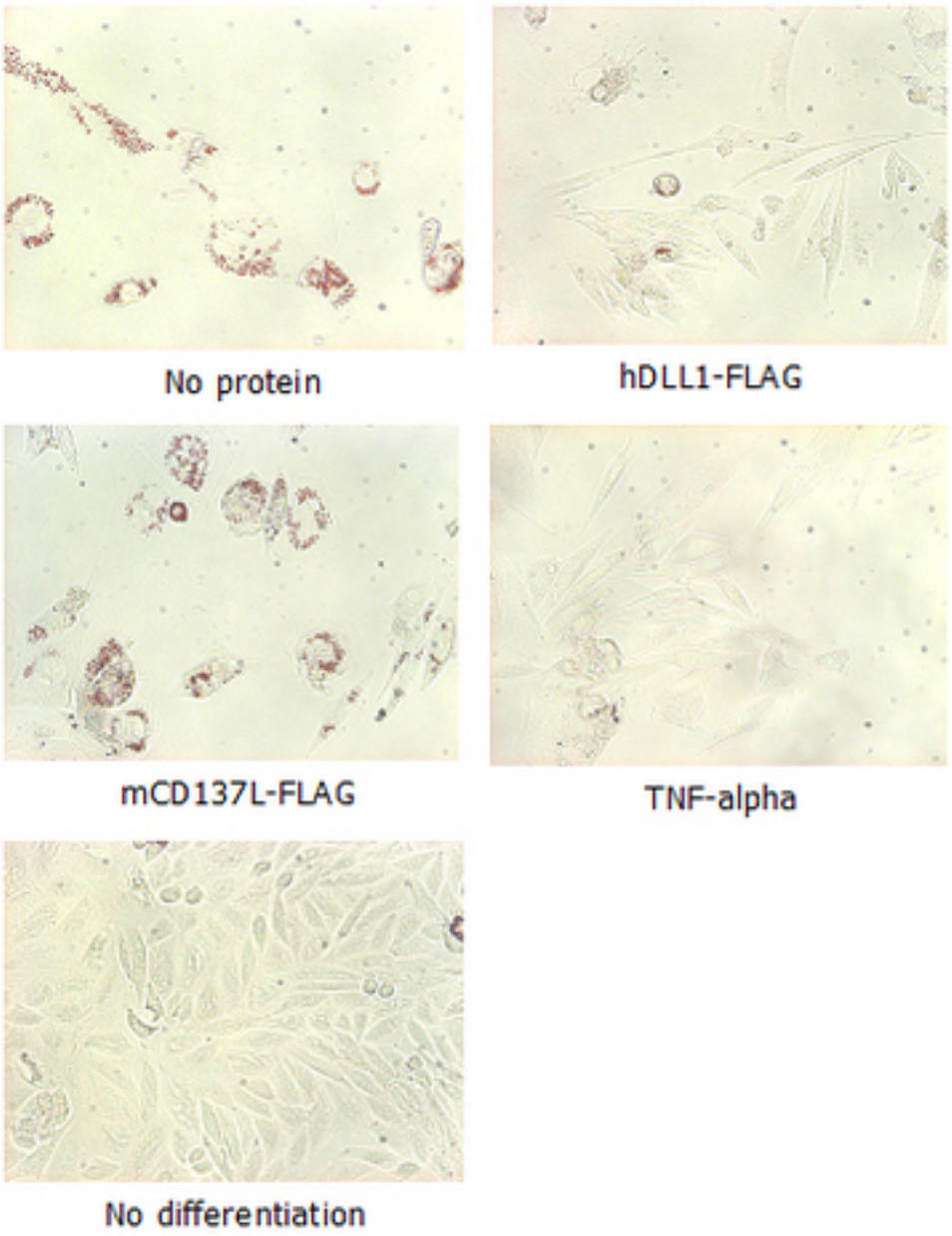

Figure 2: Adipogenesis inhibition of MSCs.
MSCs (Mesenchymal stem cells) were maintained in DMEM, supplemented with 10% fetal bovine serum, penicilin-streptomycin and glutamine. For differentiation of MSCs, MSCs were cultured in adipogenic medium which was growth medium supplemented with 1µM Dexamethasone, 0.5mM IBMX, 10µg/m Insulin, 100µM Indomethacin (day 1). Medium was changed every 3 days. Staining with Oil Red O was typically performed on day 30. For negative controls, TNF-α (20ng/ml) was added. To immobilize Notch ligands on the plastic surface of the culture plates, plates were incubated with a solution of hDLL4-Fc (Prod. No. AG-40A-0077) (5µg/ml) or mCD137-Fc (5µg/ml) in PBS for 2 hours at 37°C. Plates were then used to differentiate MSCs.

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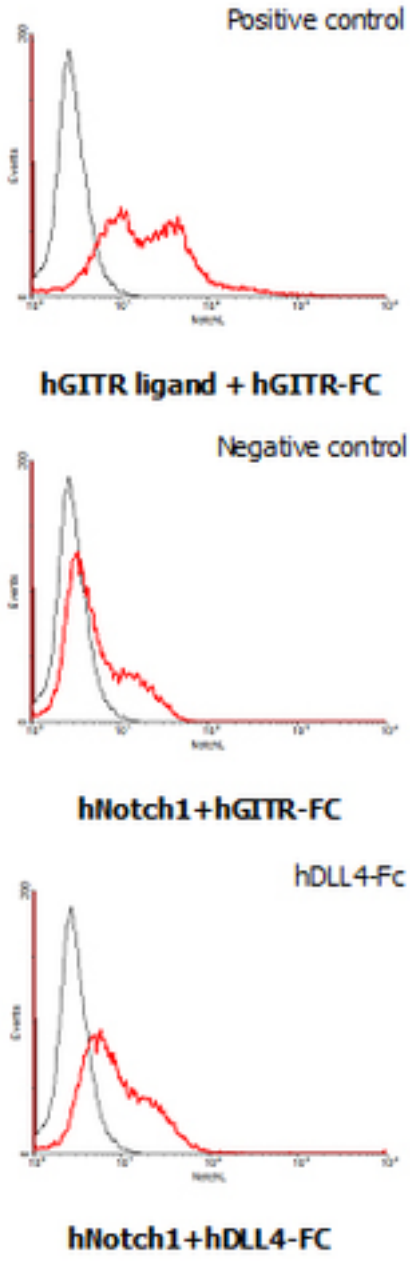

Figure 3: Interaction of human Notch1 with human DLL4. HEK293 cells transfected with a human Notch1 or a human GITR ligand expressing vector were incubated with 25µg/ml of human GITR-Fc or human DLL4-Fc (Prod. No. AG-40A-0077). Cells were stained with anti-human IgG(Fc specific) FITC conjugate for DLL4-Fc binding.

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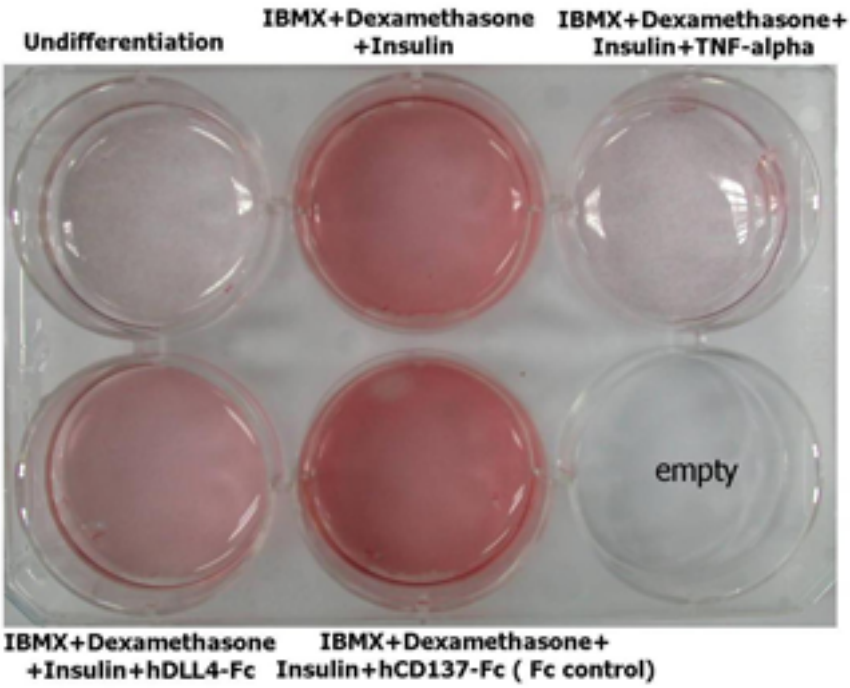

Figure 4: Adipogenesis inhibition of 3T3L-1 cells.

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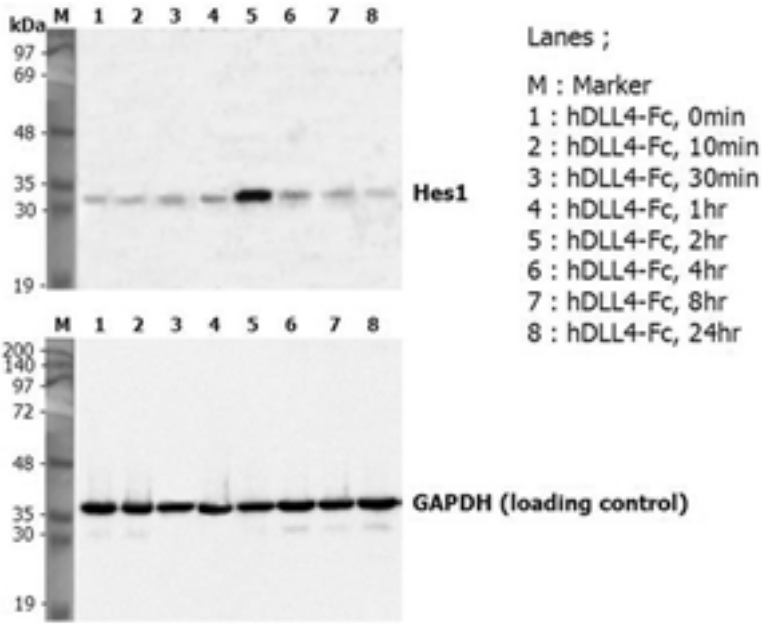

Figure 5: Induction of Hes-1 with the treatment of recombinant human DLL4-Fc (Prod. No. AG-40A-0077). A mouse preadpipocyte cell line, 3T3L1, was stimulated with 5µg/ml of human DLL4-Fc as in indicated time points and each cell lysate was prepared and subjected to western blot by using anti-mouse Hes1 or GAPDH.

Figure 6: 50µg of cell lysates derived from hDLL4-Fc or non-treated 3T3L1 cells, which had been either differentiated or undifferentiated, were subjected to western blot by using a mouse adiponectin antibody.

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