DLL1, Soluble (human) ELISA Kit

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Instruction Manual

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FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

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Introduction

The Notch signaling is essential for appropriate cell differentiation and cell fate decisions. DLL1 is a human homolog of the Drosophila Notch ligand Delta. A cDNA encoding DLL1 was isolated by a PCR cloning approach utilizing a primer pair whose sequences were derived from the Delta gene. DLL1 is the 723-amino acid type I transmembrane protein, which is 88% identical to the mouse Dll1 protein, having a DSL domain followed by 8 tandem EGF-like repeats and a short cytoplasmic C-terminal region (1). Engagement of Notch with DLL1 was shown to inhibit the differentiation of muscle, myeloid, or lymphoid progenitors (2-4). It has been shown that DLL1 is subjected to ADAM metaloproteases (ADAM10 & ADAM12) and presenilin-dependent intramembranous gamma-secretase processing, resulting in the production of soluble extracellular domain and intracellular domain (5-7). ADAM-mediated shedding of DLL1 in a subset of cells during myogenic differentiation in vitro seems to contribute to downregulation of Notch signaling in neighboring cells and facilitates their progression into differentiation such that the proteolytic processing of DLL1 helps achieve an asymmetry in Notch signaling in initially equivalent myogenic cells and helps sustain the balance between differentiation and self-renewal. Since obesity is a consequence of increase in adipocyte proliferation, DLL1 could be also involved in differentiation and self-renewal of adipocyte stem cells. Depending on metabolic status, shedding of DLL1 could be affected. Thus, measurement of soluble DLL1 in serum or plasma likely provides a novel indication of disease status in metabolic dysfunctions.

Assay Principles

This kit is an enzyme-linked immunosorbent assay (ELISA) for quantitative determination of soluble DLL1 in human serum, plasma or cell culture supernatants.

A monoclonal antibody specific for human soluble DLL1 has been pre-coated onto 96 well microplate. Standards and samples are pipetted into the wells and any soluble DLL1 present is bound by immobilized antibody. Bound soluble DLL1 is captured by purified anti-human DLL1 polyclonal antibody. HRP conjugated anti-rabbit IgG is added. After washing, a substrate solution is added. The colors develop in proportion to the bounded soluble DLL1 quantity. The color development is stopped and the intensity of color is measured.

Kit Components

1) Antibody coated 96-well plate, 12 X 8-well strips
2) 5x Wash concentrate, 100 ml
3) 5x Diluent, 50 ml
4) Secondary antibody, 12 ml
5) 100x Detector, 150 µl
6) Standard, recombinant human DLL1 expressed by HEK 293 cells, 1 vial, lyophilized
7) QC sample = a positive control of human serum DLL1, 1 vial, lyophilized (For actual concentrations of QC sample, see the 'Certificate of analysis' enclosed.)
8) Substrate, 12 ml
9) Stop solution, 12 ml
10) Plate sealer, 3 sealers

Reagents Description

Antibody coated 96-well plate, 12 x 8-well strips, with absorbed monoclonal antibody against human soluble DLL1

5x Wash concentrate, buffered detergent solution, supplied as a 5x concentrate

5x Diluent, for sample and reagent dilution

1x Secondary antibody, polyclonal antibody against human soluble DLL1
100x detector, HRP conjugated rabbit IgG
Standard, 16.0 ng, 1 vial each, recombinant human soluble DLL1, lyophilized
QC sample, 1 vial each, a positive control of human serum DLL1, lyophilized
Substrate, chromogenic reagents
Stop solution, 1M H_3PO_4

Storage of Reagents

Reagent must be stored at 2-8°C when not in use. Reagents must be brought to room temperature before use. Do not expose reagents to temperatures greater than 25°C. Diluted wash solution may be stored at room temperature for up to one month.

Materials Required but not Supplied

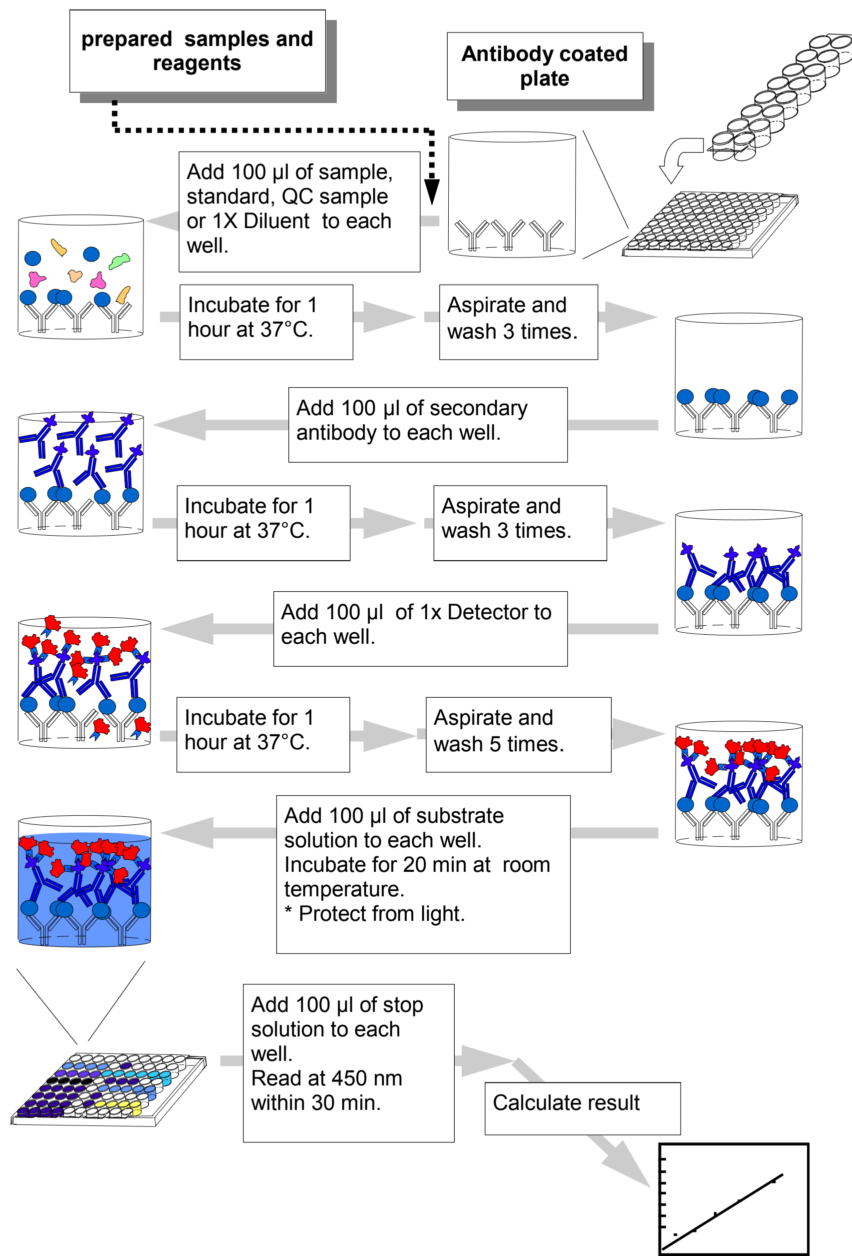
Precision single and multi-channel pipettes
Disposable pipette tips
Microtubes or equivalent for preparing dilutions
Disposable plastic containers for preparing working reagents
Reagent reservoirs
Microwell or microstrip plate reader 450 nm
Deionized water

Sample Collection and Storage

Serum Use a serum separator tube. Let samples clot at room temperature for 30 minutes before centrifugation for 20 minutes at 1,000xg. Assay freshly prepared serum or store serum in aliquot at ≤ - 20°C for later use. Avoid repeated freeze/thaw cycles.

Plasma Collect plasma using heparin, EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay freshly prepared plasma or store plasma sample in aliquot at ≤ - 20°C for later use. Avoid repeated freeze/thaw cycles.

Flow Chart of Assay Procedure



Assay Procedure

1. Preparation of Reagents

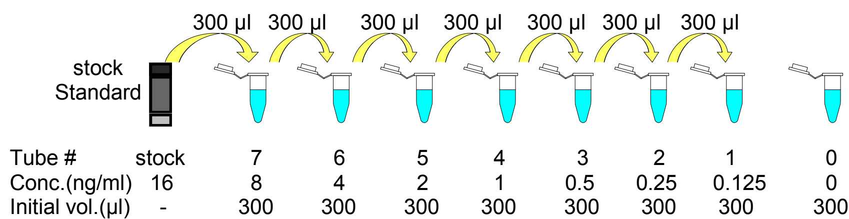
1) Allow all samples and kit components to equilibrate to room temperature (20-25°C).
2) Plan the plate configuration and create a plate map. Calculate the amount of working reagents to use.
   It is recommended that Standards and samples be run in duplicate.
3) Prepare **1X Wash Solution**. Dilute 5X Wash Concentrate 1:5 with deionized water (1 part 5X Wash Concentrate with 4 parts deionized water). The diluted 1X Wash Solution is stable for one month at room temperature.
4) Prepare **1X Diluent**. Dilute 5X Diluent 1:5 with deionized water (1 part 5X Diluent with 4 parts deionized water).
5) Prepare **1X Detector**. Dilute 100X Detector 1:100 with 1X Diluent (1 part 100X Detector with 99 parts 1X Diluent). Use the 1X Detector within one hour of preparation.
6) Warm **Substrate Solution** to room temperature before use.
7) Prepare working aliquots of the **Standard** as follows :
   When opening the lyophilized Standard, remove cap gently as the lyophilizate may have become dislodged during shipping.
   Add 1 ml of deionized water to the Standard vial to make a stock concentration of 16 ng/ml. Mix well.

A recommended dilution scheme is as follows :

A) Label 8 microcentrifuge tubes #0-7. Add 300 µl of the 1X Diluent to the microcentrifuge tubes # 0-7.

B) Add 300 µl of the stock Standard solution to tube # 7 and vortex. This is Standard tube # 7 with a concentration of 8 ng/ml.

C) Standards # 6 to # 1 are then prepared by performing a 1:2 dilution of the preceding standard. Do not add any standard to the tube # 0.



Tube #	stock	7	6	5	4	3	2	1	0
Conc.(ng/ml)	16	8	4	2	1	0.5	0.25	0.125	0
Initial vol.(µl)	-	300	300	300	300	300	300	300	300

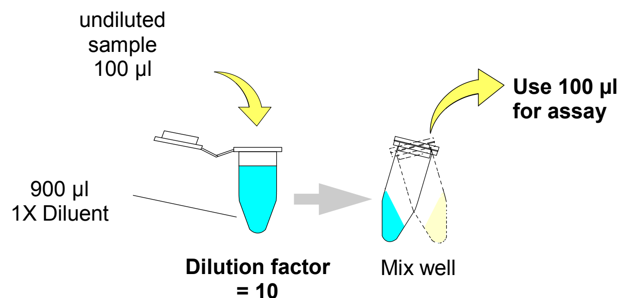
8) Reconstitute QC sample in 1 ml of deionized water. Mix well.

2. Sample Preparation

1) Dilute samples 1:10 with 1X Diluent (example, 100 µl sample plus 900 µl 1X Diluent; dilution factor=10) and mix well.

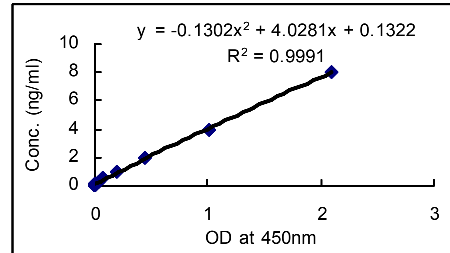
* If samples fall the outside range of assay, a lower or higher dilution may be required.

2) Use 100 µl of the final diluted sample for ELISA.



3. Experiment procedure

1) Remove the appropriate number of microwell strips from the sealed foil pouch.
2) Pipette 100 µl of standard # 0 to # 7, the reconstituted QC sample and diluted sample into the antibody-coated plate according to the plate configuration. Use a new pipette tip for each standard or sample.
3) Incubate at 37°C for 1 hour.
4) Remove the solution and wash 3 times with 300 µl of 1X Wash Solution to each well.
5) Add 100 µl Secondary Antibody to each well.
6) Incubate at 37°C for 1 hour.
7) Remove the solution and wash 3 times with 300 µl of 1X Wash Solution to each well.
8) Add 100 µl 1X Detector to each well.
9) Incubate at 37°C for 1 hour.
10) Remove the solution and wash 5 times with 300 µl of 1X Wash Solution to each well.
11) Add 100 µl of the Substrate Solution to each well.
12) Incubate at room temperature for 20 min.
    * Protect from light.
13) Using the multi-channel pipette, add 100 µl Stop Solution to each well.
14) Read at 450 nm.
15) Subtract the absorbance of the blank from the readings for each standard and sample.



16) Construct a standard curve by plotting the known concentrations (Y) of standard versus the absorbances (X) of standard. A measurable range is typically shown between 0.125 ng/ml and 8 ng/ml.
17) Calculate the soluble DLL1 concentrations of samples by interpolation of the regression curve formula as shown above in a form of a quadratic equation
18) The soluble DLL1 concentrations calculated must be multiplied by dilution factor [see **2. Sample Preparation**] to obtain the concentrations of the undiluted samples.

Performance Characteristics

1) Sensitivity : 120 pg/ml

2) Precision

a. Intra-Assay (precision within an assay)
6 samples were tested 4 times to assess intra-assay precision.

Sample	Mean (ng/ml)	SD (ng/ml)	CV (%)
1	25.00	0.61	2.44
2	26.37	0.87	3.29
3	21.94	1.19	5.40
4	28.49	1.00	3.52
5	29.56	0.88	2.99
6	27.63	1.22	4.41

b. Inter-Assay (precision between assays)
6 samples were tested 4 times to assess inter-assay precision.

Sample	Mean (ng/ml)	SD (ng/ml)	CV (%)
1	26.26	1.78	6.77
2	27.71	2.29	8.26
3	30.34	2.12	7.00
4	14.76	1.20	8.14
5	30.03	1.68	5.60
6	28.84	1.14	3.95

3) Specificity

a. No cross reaction with mouse and rat sera
b. Cross Reactivity

Analyte	Max. Conc. (ng/ml)	Cross Reactivity (%)
Human DLL1	4	100
Human DLL4	40	N. R.
Human DNER	40	N. R.
Human DLK1	40	N. R.
Human Jagged1	40	N. R.
Human ANGPTL3	40	N. R.
Human FTO	40	N. R.
Human ACE2	40	N. R.
Human FGF21	40	N. R.
Human Adiponectin	40	N. R.
Human Resistin	40	N. R.
Human Vaspin	40	N. R.
Human GPX3	40	N. R.
Human Clusterin	40	N. R.
Human IL-33	40	N. R.
Human FABP4	40	N. R.
Human Leptin	40	N. R.
Human RBP4	40	N. R.
Mouse Adiponectin	40	N. R.

N. R. : No Cross-reactivity

4) Recovery

The recovery of soluble DLL1 spiked to three different levels in three different human serum samples throughout the range of assay was evaluated.

Sample No.	Average recovery (%)	Range (%)
1	101.07	95-105
2	102.05	95-105
3	106.15	100-110

5) Linearity - Effect of Serum Dilution

To assess the linearity of the assay, three serum samples were first diluted as indicated below prior to sample preparation as described in the protocol.

Sample No.	Serum Dilution	Expected (ng/ml)	Observed (ng/ml)	% Of Expected
1	1:5	33.16	33.16	100
	1:10	16.58	17.22	103.89
2	1:5	39.38	39.38	100
	1:10	19.69	20.28	102.98
3	1:5	37.87	37.87	100
	1:10	18.94	17.28	91.24

% of expected = observed / expected x 100%

6) Comparison of serum samples with plasma samples

Sample No.	Serum (ng/ml)	Plasma (ng/ml)		
		Sodium citrate	EDTA	Heparin
1	21.31	11.39	19.46	17.40
2	20.24	10.25	20.83	14.52
3	13.71	7.94	14.89	14.70

References

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	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Troubleshooting Guide

Problem	Possible Cause	Solution
No signal or weak signal	Omission of key reagent	Check that all reagents have been added in the correct order.
	Washes too stringent	Use an automated plate washer if possible.
	Incubation times inadequate	Incubation times should be appropriate for the system.
	Plate reader settings not optimal	Verify the wavelength and filter setting in the plate reader.
	Incorrect assay temperature	Use recommended incubation temperature. Bring substrates to room temperature before use.
High background	Concentration of detector too high	Use recommended dilution factor.
	Inadequate washing	Ensure all wells are filling wash buffer and are aspirated completely.
Poor standard curve	Wells not completely aspirated	Completely aspirate wells between steps.
	Reagents poorly mixed	Be sure that reagents are thoroughly mixed.
Unexpected results	Omission of reagents	Be sure that reagents were prepared correctly and added in the correct order.
	Dilution error	Check pipetting technique and double-check calculations.
	Technique problem	Proper mixing of reagents and wash steps are critical.

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