



PRODUCT INFORMATION & ELISA MANUAL

DLL1 Antibody Pair [HRP]

NBP2-79662

Sample Insert for reference use only

Matched Antibody Pair utilized in an Enzyme-linked
Immunosorbent Assay for quantitative detection of
Human DLL1.

For research use only.

Not for diagnostic or therapeutic procedures.

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Novus kits are guaranteed for 6 months from date of receipt

BACKGROUND

Delta-like protein 1(DLL1), also known as Delta1, a single-pass type I membrane protein which contains one DSL domain and eight EGF-like domains, acts as a ligand for Notch receptors, and positively regulates T-cell development. DLL1 is proteolytically processed in a similar manner to the Notch receptor, and it has been speculated to participate in bidirectional signaling. 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. Interactions between DLL1 and Notch in trans activate the Notch pathway, whereas DLL1 binding to Notch in cis inhibits Notch signaling. DLL1 undergoes proteolytic processing in its extracellular domain by ADAM10. It had been demonstrated that DLL1 represents a substrate for several other members of the ADAM family. In co-transfected cells, DLL1 is constitutively cleaved by ADAM12, and the N-terminal fragment of DLL1 is released to medium. ADAM12-mediated cleavage of DLL1 is cell density-dependent, takes place in cis orientation, and does not require the presence of the cytoplasmic domain of ADAM12. Full-length DLL1, but not its N- or C-terminal proteolytic fragment, co-immunoprecipitates with ADAM12. By using a Notch reporter construct, we show that DLL1 processing by ADAM12 increases Notch signaling in a cell-autonomous manner. Furthermore, ADAM9 and ADAM17 have the ability to process DLL1. In contrast, ADAM15 does not cleave DLL1, although the two proteins still co-immunoprecipitate with each other. During fetal development, DLL1 is an essential Notch ligand in the vascular endothelium of large arteries to activate Notch1 and maintain arterial identity. DLL1-Notch signaling was required for VEGF receptor expression in fetal arteries.

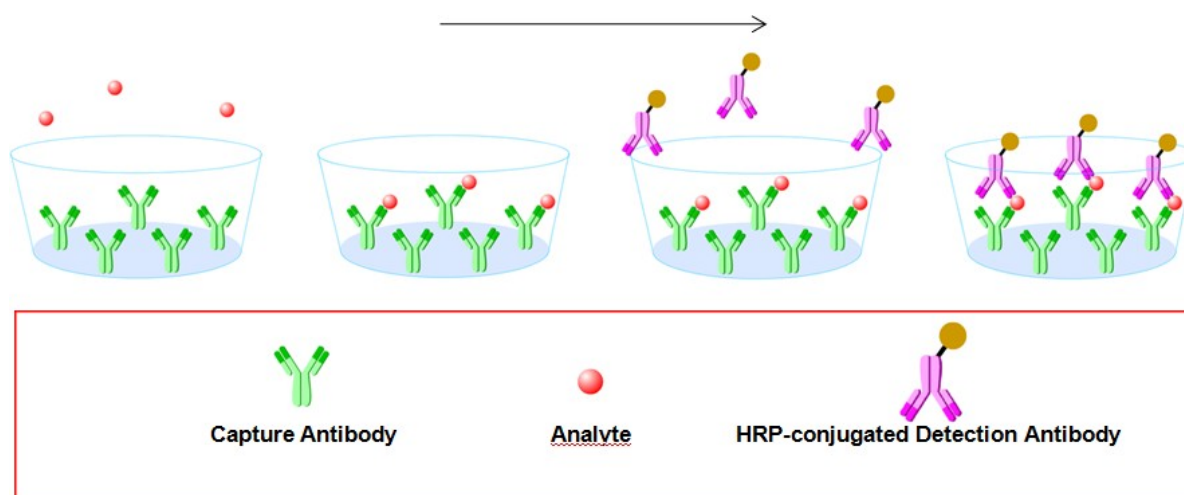
PRINCIPLE OF THE TEST

The Novus Biologicals DLL1 Antibody Pair [HRP] is a solid phase sandwich ELISA (Enzyme-Linked Immunosorbent Assay). It utilizes a monoclonal antibody specific for DLL1 coated on a 96-well plate. Standards and samples are added to the wells, and any DLL1 present binds to the immobilized antibody. The wells are washed and a horseradish peroxidase conjugated mouse anti-DLL1 monoclonal antibody is then added, producing an antibody-antigen-antibody “sandwich”. The wells are again washed and TMB substrate solution is loaded, which produces color in proportion to the amount of DLL1 present in the sample. To end the enzyme reaction, the stop solution is added and absorbances of the microwell are read at 450 nm.

INTENDED USE

- ◆ The Human DLL1 Antibody Pair [HRP] is for the quantitative determination of Human DLL1.
- ◆ This DLL1 Antibody Pair [HRP] contains the basic components required for the development of sandwich ELISAs.

ASSAY PROCEDURE SUMMARY



This antibody pair has been configured for research use only and is not to be used in diagnostic procedures.

MATERIALS PROVIDED

Bring all reagents to room temperature before use.

Capture Antibody – 0.4 mg/mL of mouse anti-DLL1 monoclonal antibody. Dilute to a working concentration of 2.0 µg/mL in CBS before coating.

Detection Antibody – 0.5 mg/mL mouse anti-DLL1 monoclonal antibody conjugated to horseradish-peroxidase (HRP). Dilute to working concentration of 0.5 µg/mL in detection antibody dilution buffer before use.

Standard – Each vial contains 100 ng of recombinant DLL1. Reconstitute standard powder with 1 mL detection antibody dilution buffer. After reconstitution, store at -20°C to -80°C in a manual defrost freezer. A seven-point standard curve using 2-fold serial dilutions in sample dilution buffer, and a high standard of 3000 pg/mL is recommended.

SOLUTIONS REQUIRED

CBS - 0.05M Na₂CO₃, 0.05M NaHCO₃, pH 9.6, 0.2 µm filtered

TBS - 20 mM Tris, 150 mM NaCl, pH 7.4

Wash Buffer - 0.05% Tween20 in TBS, pH 7.2 - 7.4

Blocking Buffer - 2% BSA in Wash Buffer

Sample dilution buffer - 0.1% BSA in wash buffer, pH 7.2 - 7.4, 0.2 µm filtered

Detection antibody dilution buffer - 0.5% BSA in wash buffer, pH 7.2 - 7.4, 0.2 µm filtered

Substrate Solution : To achieve best assay results, fresh substrate solution is recommended

Substrate stock solution - 10mg / ml TMB (Tetramethylbenzidine) in DMSO

Substrate dilution buffer - 0.05M Na₂HPO₄ and 0.025M citric acid ; adjust pH to 5.5

Substrate working solution - For each plate dilute 250 µl substrate stock solution in 25ml substrate dilution buffer and then add 80 µl 0.75% H₂O₂, mix it well

Stop Solution - 2 N H₂SO₄

PRECAUTION

The Stop Solution suggested for use with this antibody pair is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

STORAGE

Capture Antibody: Aliquot and store at -20°C to -80°C for up to 6 months from date of receipt. Avoid repeated freeze-thaw cycles.

Detection Antibody: Protect it from prolonged exposure to light. Aliquot and store at -20°C to -80°C and for up to 6 months from date of receipt. Avoid repeated freeze-thaw cycles.

Standard: Store lyophilized standard at -20°C to -80°C for up to 6 months from date of receipt. Aliquot and store the reconstituted standard at -80°C for up to 1 month. Avoid repeated freeze-thaw cycles.

GENERAL ELISA PROTOCOL

Plate Preparation

1. Dilute the capture antibody to the working concentration in CBS. Immediately coat a 96-well microplate with 100µL per well of the diluted capture antibody. Seal the plate and incubate overnight at 4°C.
2. Aspirate each well and wash with at least 300µl wash buffer, repeating the process two times for a total of three washes. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining wash buffer by inverting the plate and blotting it against clean paper towels.
3. Block plates by adding 300 µL of blocking buffer to each well. Incubate at room temperature for a minimum of 1 hour.
4. Repeat the aspiration/wash as in step 2. The plates are now ready for sample addition.

Assay Procedure

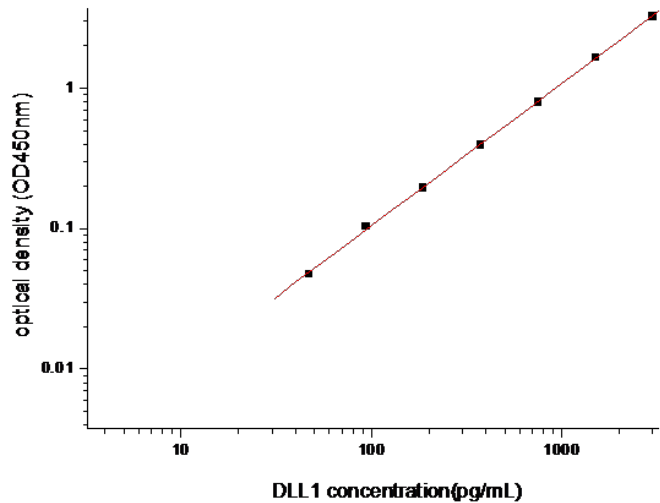
1. Add 100 µL of sample or standards in sample dilution buffer per well. Seal the plate and incubate 2 hours at room temperature.
2. Repeat the aspiration/wash as in step 2 of plate preparation.
3. Add 100 µL of the detection antibody, diluted in antibody dilution buffer, to each well. Seal the plate and incubate 1 hour at room temperature.
4. Repeat the aspiration/wash as in step 2 of plate preparation.
5. Add 200 µL of substrate solution to each well. Incubate for 20 minutes at room temperature (**if substrate solution is not as requested, the incubation time should be optimized**). Avoid placing the plate in direct light.
6. Add 50 µL of stop solution to each well. Gently tap the plate to ensure thorough mixing.
7. Determine the optical density of each well immediately, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

- Calculate the mean absorbance for each set of duplicate standards, controls and samples. Subtract the mean zero standard absorbance from each.
- Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.
- To determine the concentration of the unknowns, find the unknowns' mean absorbance value on the y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the x-axis and read the concentration. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
- Alternatively, computer-based curve-fitting statistical software may also be employed to calculate the concentration of the sample.

TYPICAL DATA

This standard curve is only for demonstration purposes. A standard curve should be generated for each assay.



Concentration (pg/ml)	Zero standard subtracted OD
0	0.000
46.875	0.047
93.75	0.104
187.5	0.195
375	0.395
750	0.798
1500	1.663
3000	3.248

PERFORMANCE CHARACTERISTIC

SENSITIVITY

The minimum detectable dose of Human DLL1 was determined to be approximately 46.875 pg/ml. This is defined as at least three times standard deviations above the mean optical density of 10 replicates of the zero standard.

TROUBLE SHOOTING

Problems	Possible Sources	Solutions
No signal	Incorrect or no Detection Antibody was added	Add appropriate Detection Antibody and continue
	Substrate solution was not added	Add substrate solution and continue
	Incorrect storage condition	Check if the kit is stored at recommended condition and used before expiration date
Poor Standard Curve	Standard was incompletely reconstituted or was inappropriately stored	Aliquot reconstituted standard and store at -80 °C
	Imprecise / inaccurate pipetting	Check / calibrate pipettes
	Incubations done at inappropriate temperature, timing or agitation	Follow the general ELISA protocol
	Background wells were contaminated	Avoid cross contamination by using the sealer appropriately
Poor detection value	The concentration of antigen in samples was too low	Enriching samples to increase the concentration of antigen
	Samples were ineffective	Check if the samples are stored at cold environment. Detect samples in timely manner
High Background	Insufficient washes	Use multichannel pipettes without touching the reagents on the plate
		Increase cycles of washes and soaking time between washes
	TMB Substrate Solution was contaminated	TMB Substrate Solution should be clear and colorless prior to addition to wells
	Materials were contaminated.	Use clean plates, tubes and pipettes tips
Non-specificity	Samples were contaminated	Avoid cross contamination of samples
	The concentration of samples was too high	Try higher dilution rate of samples

ELISA Plate Template

[illegible]

Human DLL1 Antibody Pair
[HRP]
Notes