Human DLL1 AccuSignal™ ELISA Kit

AccuSignal high sensitivity sandwich ELISA (Enzyme-Linked Immunosorbent Assay) Kit is *an in vitro* enzyme-linked immunosorbent assay designed for the quantitative detection of Human DLL1 in cell culture supernatants, serum, plasma (heparin, EDTA) and saliva.

I. KIT COMPONENTS

Human DLL1 AccuSignal™ ELISA Kit			
Component	Catalog Number	Description	Size
Antibody Coated ELISA Plate	KOA0752A	96-well plate pre-coated with anti-Human DLL1 antibody	1
Target Protein Standard	KOA0752B	lyophilized recombinant Human DLL1	2 vials (10 ng/vial)
Biotinylated Detection Antibody	KOA0752C	biotinylated anti-Mouse DLL1 antibody	130 µl (dilution 1:100)
ABC Complex	KAB0101	Avidin-Biotin-Peroxidase Complex (ABC)	130 µl (dilution 1:100)
Sample Diluent	KAE0101	sample diluent buffer	30 ml
Antibody Diluent	KAF0101	antibody diluent buffer	12 ml
ABC Diluent	KAG0101	ABC diluent buffer	12 ml
Substrate Solution	KAC0101	TMB color developing reagent	10 ml
Stop Solution	KAD0101	TMB stop solution	10 ml
Adhesive Cover	-	Adhesive cover	4

II. STORAGE CONDITIONS

Store vials at 4°C prior to opening. Centrifuge product if not completely clear after standing at room temperature. This product is stable for 6 months at 4°C as an undiluted liquid. Dilute only prior to immediate use. For extended storage freeze at -20°C or below for 12 months. Avoid cycles of freezing and thawing.

III. INTRODUCTION

Human DLL1 ELISA Kit is based on standard sandwich enzyme-linked immune-sorbent assay technology. This assay employs a monoclonal antibody specific for DLL1 pre-coated onto 96-well plate. Standards (Expression system for standard: NSO; Immunogen sequence: S22-G540) and test samples are pipetted into the wells, and a biotinylated detection polyclonal antibody from goat specific for DLL1 is added to the wells. The wells are washed with PBS or TBS buffer. After washing away unbound biotinylated antibody, Avidin-Biotin-Peroxidase Complex is added to the wells and unbound conjugates are washed away with PBS or TBS buffer. HRP substrate TMB solution is added to the wells to visualize HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue color product. The addition of stop solution changes the color from blue to yellow. The density of yellow is proportional to the amount of Human DLL1 amount captured in plate.

IV. REQUIRED EQUIPMENT AND REAGENTS

- Microplate reader in standard size
- Automated plate washer
- Adjustable pipettes and pipette tips
- Clean tubes and Eppendorf tubes
- Washing buffer (neutral PBS or TBS)
  - Preparation of 0.01M TBS:
    Add 1.2g Tris, 8.5g NaCl; 450µl of purified acetic acid or 700µl of concentrated hydrochloric acid to 1000 ml distilled water and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.
  - Preparation of 0.01 M PBS:
    Add 8.5 g sodium chloride, 1.4 g Na_2HPO_4 and 0.2 g NaH_2PO_4 to 1000 ml distilled water and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

V. GENERAL CONSIDERATIONS

Please read the following instructions before starting the experiment.

- To inspect the validity of experimental operation and the appropriateness of sample dilution proportions, pilot experiment using standards and a small number of samples is recommended.
- The TMB Color Developing agent is colorless and transparent before using, contact us if it is not the case.
- Before using the Kit, briefly spin down the vials.
- For statistical reasons, we recommend both standard and samples should be assayed with a minimum of two replicates (duplicates).
- Do not let 96-well plate to dry, this will inactivate active components on plate.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Do not mix or substitute reagents or materials from other kit lots or vendors.
- In order to avoid marginal effect of plate incubation due to temperature difference (reaction may be stronger in the marginal wells), it is suggested that the diluted ABC and TMB solution be pre-warmed in 37℃ for 30 min before using.

VI. SANDWICH ELISA PROTOCOL

A. Sample Preparation

Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

- **Serum:**
  Allow the serum to clot in a serum separator tube (about 4 hours) at room temperature. Centrifuge at approximately 1000 X g for 15 min. Analyze the serum immediately or aliquot and store at -20°C.
- **Cell culture supernatants:**
  Remove particulates by centrifugation, assay immediately or aliquot and store at -20°C.

- **Plasma:**
  Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 min at 1500 x g within 30 min of collection. Assay immediately or aliquot and store at -20°C.
- **Saliva:**
  Collect saliva using a collection device without any protein binding or filtering capabilities such as a Salivette or aliquot and store at -20°C.

B. Sample Dilution

Please note that levels of the target protein may vary between different samples. Estimation of the concentration of a target protein in the sample and the optimal dilution factors for each sample must be determined by the investigator. The diluted target protein concentration should fall near the middle of the linear regime in the standard curve. Sample diluent buffer should be used for dilution of samples.

Following are the suggested guidelines for sample dilution. **The samples must be mixed well with the diluent buffer**.

- **High target protein concentration (50,000pg/ml-500,000pg/ml)**
  The working dilution should be 1:100. For example, add 1µl sample into a tube with 99µl sample diluent buffer.
- **Medium target protein concentration (5000pg/ml-50,000pg/ml)**
  The working dilution should be 1:10. For example, add 10µl sample into a tube with 90µl sample diluent buffer.
- **Low target protein concentration (78pg/ml-5000pg/ml)**
  The working dilution should be 1:2. For example, add 50µl sample into a tube with 50µl sample diluent buffer.
- **Very Low target protein concentration (0pg/ml-78pg/ml)**
  No dilution necessary, or the working dilution should be 1:2.

C. Reagent Preparation

Reconstitution of the Human DLL1 standard:

DLL1 standard solution should be prepared no more than 2 hours prior to the experiment. Two vials of DLL1 standard (1ng/vial) are provided with each kit. Use one vial/experiment.

- For preparation of 10,000pg/ml of Human DLL1 standard solution, add 1ml sample diluent buffer into one vial, keep the vial at room temperature for 10 min and mix thoroughly.
- For preparation of 5000pg/ml of Human DLL1 standard solution, add 0.5ml of above DLL1 standard solution into 0.5ml diluent buffer and mix thoroughly.
- For preparation of 2500pg/ml to 78.125pg/ml of Human DLL1 standard solutions, label 6 Eppendorf tubes with 2500pg/ml (tube#1), 1250pg/ml (tube#2), 625pg/ml (tube#3), 312.5pg/ml (tube#4), 156.25pg/ml (tube #5), and 78.125pg/ml (tube #6) respectively.
- Pipette 300µl of the sample diluent buffer into each tube. Add 300µl of the above 10,000pg/ml DLL1 standard solution into tube #1 and mix thoroughly.
- Transfer 300µl from tube #1 to tube #2 and mix thoroughly.
- Transfer 300µl from tube #2 to tube #3 and mix thoroughly, and prepare further serial dilutions.

Note: The standard solutions are best used within 2 hours. The 100ng/ml standard solution should be stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.

Preparation of biotinylated anti-Human DLL1 antibody working solution:

The solution should be prepared no more than 2 hours prior to the experiment.

- The total volume should be 0.1ml/well x (the number of wells). Prepare 100-200µl more of the solution than the total volume required to compensate for pipetting errors.
- Biotinylated anti-Human DLL1 antibody should be diluted 1:100 with the antibody diluent buffer and mixed thoroughly. For example, add 1µl Biotinylated Anti-Human DLL1 antibody to 99µl antibody diluent buffer.

Preparation of Avidin-Biotin-Peroxidase Complex (ABC) working solution:

The solution should be prepared no more than 1 hour prior to the experiment.

- The total volume should be: 0.1 ml/well x (the number of wells). Prepare 0.1-0.2 ml more of the solution than total volume required.
- Avidin-Biotin-Peroxidase Complex (ABC) should be diluted 1:100 with the ABC dilution buffer and mixed thoroughly. For example, add 1µl ABC to 99µl ABC diluent buffer.

D. Assay Procedure

ABC working solution and TMB substrate reagent should be warmed at 37°C for 30 min prior to use. Samples and reagents should be mixed thoroughly and evenly after dilution. A standard curve for DLL1 detection should be prepared for each experiment. It is recommended to determine sample fold dilution by simple estimation of DLL1 amount in the samples.

1. Aliquot 100µl per well of each Human DLL1 standard (5000pg/ml,2500pg/ml, 1250pg/ml, 625pg/ml, 312.5pg/ml, 156.25pg/ml, 78.125pg/ml) into pre-coated 96-well plate. Add 100µl of the sample diluent buffer into control wells. Add 100µl of diluted sample (Mouse cell culture supernatants, serum, plasma {heparin, EDTA} or saliva into appropriate wells). See "Sample Dilution Guideline" above for details. **It is recommended to assay all standards, controls and samples in duplicate.**
2. Seal the plate with provided adhesive plastic cover and incubate at 37°C for 90 min.
3. Remove the cover and discard the solution. Invert the plate and blot it against clean paper towels or other absorbent material. Do NOT let the wells dry completely at any time.
4. Add 100µl of biotinylated anti-Human DLL1 antibody working solution to each well, seal the plate with adhesive plastic cover and incubate at 37°C for 60 min.
5. Discard the solution and wash the plate 3 times with 0.01M TBS or 0.01M PBS (wash buffer) and each time let washing buffer stay in the wells for ˜1 min. Wash by filling each well with 300µl of wash buffer using a multi-channel pipette or auto washer. Complete removal of liquid at each step is essential to good performance. (Note: for automated washing, aspirate all wells and wash THREE times with PBS or TBS buffer, overfilling the wells with PBS or TBS buffer). After the last wash, remove any remaining wash buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels or other absorbent material.
6. Add 100µl of prepared ABC working solution to each well, seal the plate and incubate at 37°C for 30 min.
7. Wash plate 5 times with 0.01M TBS or 0.01M PBS as described in step 5.
8. Add 90µl of TMB substrate reagent to each well, seal the plate and incubate for 25-30 min at 37°C in the dark. (Note: the optimal incubation time should be determined by the end user).
9. Add 100µl of stop solution to each well. The color will change to yellow immediately.

10. Read at 450 nm in a microplate reader within 30 min after adding the stop solution.

Calculations

For calculations, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The Human DLL1 concentration of the samples can be interpolated from the standard curve.

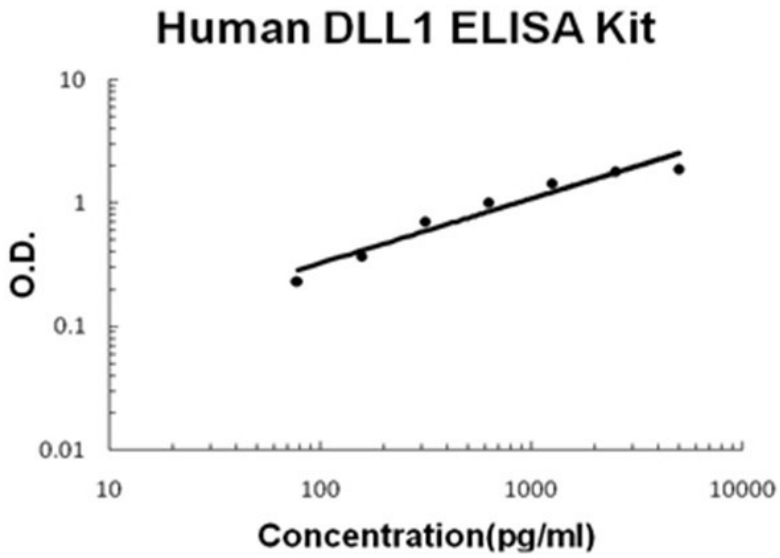
Note: if the samples measured were diluted, multiply the concentrations obtained from interpolation by the dilution factor.

VII. DATA OBTAINED FROM HUMAN DLL1

Concentration (pg/ml)	0	78	156	312	625	1250	2500	5000
O.D	0.003	0.232	0.373	0.705	1.012	1.459	1.811	1.902

(TMB reaction incubation at 37°C for 25-30min)

VIII. HUMAN DLL1 ACCUSIGNAL™ ELISA KIT STANDARD CURVE



This standard curve was generated for demonstration purpose only (A standard curve must be run with each assay).

Range	78pg/ml-5000pg/ml
Sensitivity	<10pg/ml
Specificity	Natural and recombinant Human DLL1
Cross-reactivity	There is no detectable cross-reactivity with other relevant proteins.

IX. INTRA AND INTER ASSAY PRECISION

Intra-Assay Precision (Precision within an assay): Three samples of known concentration were tested on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision between assays): Three samples of known concentration were tested in separate assays to assess inter-assay precision.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
N	16	16	16	24	24	24
Mean(pg/ml)	713	1579	3618	674	1486	3542
Standard deviation	24.9	64.74	137.5	49.8	101	269.2
CV (%)	3.5	4.1	3.8	7.4	6.8	7.6

X. ASSAY SUMMARY

- Prepare all reagents, samples and standards as instructed.
- Add standards or samples to each well used. Incubate the plate at 37°C for 90 min. Do not wash.
- Add prepared biotinylated antibody to each well. Incubate the plate at 37°C for 60 min. Wash plate 3 times with 0.01M TBS or PBS.
- Add prepared ABC working solution. Incubate the plate at 37°C for 30 min. Wash plate 5 times with 0.01M TBS or TBS.
- Add TMB developing reagent to each well. Incubate the plate at 37°C in dark for 25-30 min.
- Add TMB stop solution. Read at 450nm.

XI. TROUBLESHOOTING

Weak or No Signal

	Possible Cause	Solution
1	Problem with the standard	Use new sample Check that the standard is appropriately handled
2	Incubation time too short	Follow the exact guidelines for incubation time (If the problem persists, try incubating samples at 4°C overnight)
3	Incubation temperature too low	Ensure incubations are done at correct temperature Before proceeding
4	Incompatible sample type	Use sample that the assay is known to detect as a positive control (Include such control in your experiment)
5	Incompatible assay buffer	Ensure assay buffer is compatible with the target of interest
6	Target present below detection limit	Decrease dilution factor or concentrate samples

7	Incorrect/Insufficient/No substrate	Check the substrate identity Increase concentration or amount of substrate
8	Antibody stored at 4°C for several weeks or subjected to repeat freeze-thaw cycles	Use fresh aliquot of antibody that has been stored at -20°C or below
9	Incorrect reagents added/ prepared; Missing reagents	Check protocol, ensure correct reagents are added in proper order and prepared to correct concentrations
10	Expired/Contaminated reagents	Prepare fresh/uncontaminated reagents
11	Enzyme inhibitor present	Avoid sodium azide in HRP reactions
12	Incorrect storage of components	Check storage conditions for the kit (Kit need to be stored at 4°C)
13	Excessive plate washing	Gently pipette wash buffer (manual method) Ensure correct pressure (automatic wash system)
14	Wells dry out	Cover plate using adhesive cover at all incubation times
15	Plate read at incorrect detection wavelength	Use recommended wavelength/filter Ensure plate reader is set correctly for substrate used
16	Slow color development	Prepare substrate immediately before use Allow longer incubation time Ensure stock solution is unexpired and uncontaminated

Saturated Signal

	Possible Cause	Solution
1	High sample concentration	Use higher sample dilutions (Determine the optimal dilutions by titration assay)
2	Excessive substrate	Decrease concentration or amount of substrate: The substrate provided with the ELISA kit might require further dilution
3	Substrate color changed before use	Prepare substrate immediately before use
4	Non-specific antibody binding	Use affinity-purified antibody and preferably one that is pre-adsorbed.
5	Incubation time too long	Follow the exact guidelines (If the problem persists, try incubating samples at 4°C overnight)
6	Excessive antibody	Repeat the assay with lower antibody concentrations to find the optimal one for your experiment
7	Contaminated buffers or HRP	Prepare and use fresh buffers
8	Insufficient washing	Follow the exact guidelines At the end of each washing step, flick the plate over a sink and dry the plate on a paper towel

9	Plate adhesive cover not used or re-used	During incubations, cover plates with adhesive cover. Use a fresh cover every time the used cover is removed from the plate
10	Plate read at incorrect wavelength	Use recommended wavelength/filter Ensure plate reader is set correctly for the substrate used
11	Excess time before plate reading	Read your plate within 30 minutes after adding the substrate (If the reading is not performed within this time frame, add a stopping solution after sufficient color is developed in the plate)

High Background

	Possible Cause	Solution
1	Insufficient washing	Follow the exact guidelines At the end of each washing step, flick the plate over a sink and pat dry the plate on a paper towel
2	Excessive antibody	Repeat the assay with lower antibody concentrations to find the optimal one for your experiment
3	Excessive substrate	Decrease concentration or amount of substrate
4	Cross reactivity	Run appropriate controls
5	Non-specific antibody binding	Use affinity-purified antibody and preferably one that is pre-adsorbed
6	Insufficient Tween in buffers	Use PBS or TBS containing 0.05% Tween
7	Suboptimal salt concentration in washing buffer	Optimize salt concentration as high concentration can reduce non-specific interactions
8	Incubation temperature too high	Optimize incubation temperature for your assay (antibodies bind optimally at very specific temperature)
9	Reagents were not mixed properly	Thoroughly mix all reagents and samples before pipetting solutions into wells
10	Blanks contaminated with samples	Change pipette tips when switching between blanks and samples
11	Sample contaminated with enzymes	Test samples with substrate alone to check for contaminating enzymes
12	Contaminated TMB substrate	Use a clean container to check that the substrate in not contaminated (TMB substrate should be clear and colorless before adding to wells)

13	Substrate exposed to light	Carry out substrate incubation in dark
14	Evaporation of solution from well during incubation	Always incubate with a cover on the plate
15	Incubation time too long	Follow the exact guidelines for incubation times (If the problem persists, try incubating samples at 4°C overnight)
16	Incorrect standard curve dilutions	Check pipetting techniques Check calculations
17	Unstopped color development	Use Stopping solution to prevent over-development
18	Excessive time lapsed before plate reading	Read your plate within 30 minutes after adding the substrate (If the reading is not performed within this time frame, add a stopping solution after sufficient color is developed in the plate)
19	Incorrect plate reading setting	Use recommended wavelength/filter Ensure plate reader is set correctly for the substrate used

Low Sensitivity

	Possible Cause	Solution
1	Improper storage of ELISA kit	Store all reagents as recommended
2	Insufficient target	Reduce sample dilution or concentrate sample
3	Inactive substrate	Ensure reporter enzyme has the expected activity
4	Insufficient substrate	Increase concentration or amount of substrate
5	Incompatible sample type	Include positive control in your experiment
6	Interfering ingredients in buffers and sample	Check reagents for any interfering chemicals, e.g. sodium azide in antibodies inhibit HRP enzyme; EDTA used as anti-coagulant for plasma collection inhibits enzymatic reactions
7	Mixing or substituting reagents from different kits	Avoid mixing components from different kits
8	Incorrect plate reading setting	Use recommended wavelength/filter Ensure plate reader is set correctly for the substrate used

Poor Standard Curve Generation

	Possible Cause	Solution
1	Improper standard solution	Confirm dilutions are done correctly Prepare new standard curve as appropriate
2	Standard improperly reconstituted	Briefly spin vial before opening Inspect for undissolved material after reconstituting
3	Standard degraded	Store and handle standard as recommended Prepare standards no more than two hours before use
4	Pipetting error	Use calibrated pipettes and proper pipetting technique
5	Insufficient washing	Follow the exact guidelines At the end of each washing step, flick the plate over a sink and pat dry the plate on a paper towel
6	Poorly mixed reagents	Thoroughly mix reagents
7	Plates stacked during incubation	Keep plates separated if not using rotating plates

Poor Replicate Data

	Possible Cause	Solution
1	Bubbles in wells	Ensure no bubbles are present prior to reading the plate
2	Insufficient washing of wells	Carefully wash wells Check that all ports of the plate washer are unobstructed
3	Incomplete reagent mixing	Ensure all reagents are mixed thoroughly
4	Inconsistent pipetting	Use calibrated pipettes and proper pipetting techniques Use a new cover every time the used cover is removed from the plate
5	Inconsistent sample preparation or storage	Ensure consistent sample preparation and optimal sample storage (e.g. minimize freeze/thaw cycles)
6	Particulates in samples	Remove the particulates by centrifugation
7	Cross-well contamination	Ensure plate covers and pipette tips are not contaminated with reagents
8	Edge effect (higher or lower OD in peripheral wells than in central wells)	Ensure plates and reagents are kept at temperatures as instructed During incubation, seal the plate completely and avoid stacking plates

Inconsistent Assay-to-Assay Results

	Possible Cause	Solution
1	Insufficient washing of wells	Carefully wash wells Check that all ports of the plate washer are unobstructed

2	Varied incubation temperatures	Adhere to recommended incubation temperature
3	Variation in protocol	Adhere to the same protocol from experiment to experiment
4	Plate cover not used or re-used	During incubations, cover plates with plate cover Use a new cover every time the used one is removed
5	Incorrect dilutions	Confirm dilutions are done correctly for standard solutions Prepare new standard curve as appropriate
6	Contaminated buffers	Prepare and use fresh buffers

Slow Color Development

	Possible Cause	Solution
1	Substrates too old, contaminated or used at incorrect pH	Prepare fresh substrates at correct pH
2	Expired/Contaminated solutions	Prepare fresh reagents before use
3	Incorrect incubation temperature	Ensure plates and reagents are kept at temperatures as instructed During incubation, seal the plate completely and avoid stacking plates
4	Low antibody concentration	Repeat the assay with higher antibody concentrations to find the optimal one for your experiment

Plate Imaging Problem

	Possible Cause	Solution
1	Oversaturated image after acquisition	Use full resolution image to analyze results (Do not use jpeg or other compressed formats)
2	Blurry spots in images	Re-focus your camera before taking a new image
3	Repeated pixel values or rectangular spots	Use lower bin size, higher image resolution and/or lossless file type
4	Flat standard in images	Reduce acquisition time

XII. RELATED PRODUCTS

Component	Catalog #	Size
10x TBS pH 7.5	MB-012	1000 ml
10x TTBS pH 7.5	MB-013	1000 ml
10X PBS pH 7.2	MB-008	1000 ml
10x PBST pH 7.2	MB-075-1000	1000 ml