HUMAN SOLUBLE DELTA-LIKE PROTEIN 4 (DLL4) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN SOLUBLE DLL4 CONCENTRATIONS IN SERUM, EDTA PLASMA, CELL CULTURES

ALWAYS REFER TO LOT SPECIFIC PROTOCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ BEFORE USING THIS PRODUCT.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

PRODUCT INFORMATION:
THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	**HUMAN SOLUBLE DELTA-LIKE PROTEIN 4 (DLL4) ELISA KIT**
Catalog No.	SK00538-01
Lot No.:	20114685
Formulation	96 T
Standard range	31.25 – 2000 pg/mL
Sensitivity	15 pg/mL
Sample Volume	100 µL
Dilution Factor	**Optimal dilutions should be determined by each laboratory for each application**
Sample Type	Serum, EDTA Plasma, Cell Cultures
Specificity	Human DLL4
Calibration	Human Soluble DLL4 Rec. (HEK293)
Intra-assay Precision	4 - 6%
Inter-assay Precision	4 - 9%
Storage	2 – 8°C for 6 months. More detail check page 2
This kit contains sufficient materials to run approximately 35-40 samples duplicated provided that assay is run according to protocol.	

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DESCRIPTION

This Human Soluble Delta-Like Protein 4 (DLL4) ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human DLL4 from serum, EDTA plasma, cell cultures in a sandwich ELISA format.

This immunoassay contains recombinant human DLL4 and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural human DLL4 samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with a monoclonal antibody specific for human DLL4. The capture antibody can bind to the human DLL4 in the standard and samples. After washing the plate of any unbound substances, another monoclonal antibody-HRP conjugate against human DLL4 is added to the wells. After the last wash to remove any unbound enzyme, a substrate solution is added to the wells and color develops in direct proportion to the amount of human DLL4 bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

PROCEDURAL LIMITATIONS

_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_This ELISA kit should not be used beyond the expiration date on the kit label.
_Do not mix reagents with those from other lots or sources.
_It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.
_Each laboratory must determine the optimal dilution factors for the samples being assayed.
_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.
_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
DLL4 Microplate – 96 well microplate coated with a monoclonal antibody specific for human DLL4.	**538-01-01**	**1 plate**
DLL4 Standard – 4000 pg/vial of lyophilized recombinant human DLL4.	**538-01-02**	**1 vial**
Detection Antibody-HRP Conjugate – 110 µL/vial of 100-fold concentrated solution of monoclonal antibody conjugated to HRP against human DLL4.	**538-01-03**	**1 vial**
Positive Control – one vial of lyophilized recombinant human DLL4.	**538-01-04**	**1 vial**
Dilution Buffer - 45 mL of buffered solution with preservative.	**DB10**	**1 bottle**
Wash Buffer 20X- 25mL of 20-fold concentrated buffered surfactant with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution - 11 mL of TMB substrate solution.	**TMB03**	**1 bottle**
Stop Solution - 11 mL of 0.25M HCl.	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1 piece**
Plastic Pouch	**P01**	**1 piece**

STORAGE

Unopened Kit: Store at 2 – 8°C for up to 6 months. For longer storage up to 10 months, unopened Standard, Positive Control and Dilution Buffer (DB10) should be stored at -20°C or -70°C. **Detection Antibody-HRP Conjugate and TMB substrate solution should be stored only at 2 ~ 8°C.** Do not use kit past expiration date.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (350 – 400 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Other sample types, such as serum and plasma, need to be validated prior to use.

Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Human Serum or EDTA plasma does not require any dilution.
Optimal dilutions should be determined by each laboratory for each application with a pretest.
Use polypropylene test tubes.

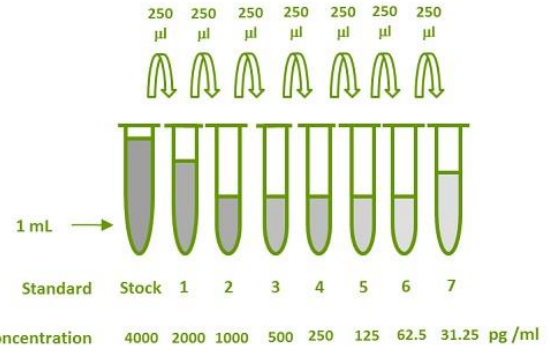
REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 25 mL of Wash Buffer Concentrate 20X into deionized or distilled water (475 mL) to prepare 500 mL of 1x Wash Buffer.

DLL4 Standard - Reconstitute the DLL4 standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 4000 pg/mL. Allow the stock standard to sit for at least 15 minutes with gentle agitation until completely dissolved prior to making standard dilutions (see below). Mix each tube thoroughly before the next transfer. The **2000 pg/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1 ml	4000 pg/ml
# 1	250 µl of stock	250 µl	2000 pg/ml
# 2	250 µl of 1	250 µl	1000 pg/ml
# 3	250 µl of 2	250 µl	500 pg/ml
# 4	250 µl of 3	250 µl	250 pg/ml
# 5	250 µl of 4	250 µl	125 pg/ml
# 6	250 µl of 5	250 µl	62.5 pg/ml
# 7	250 µl of 6	250 µl	31.25 pg/ml



Positive Control - Reconstitute the Positive Control with 1.0 mL of Dilution Buffer.

Detection Antibody-HRP Conjugate – Freshly Pipette **9.9 mL** of Dilution Buffer into a 15 mL centrifuge tube and transfer **100 µL of 100-fold** concentrated stock solution to prepare working solution **(protect from light)**. **DO NOT FREEZE.**

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Add 100 µl per well of Dilution Buffer to Blank wells.
3. Add 100 µl per well of standard dilutions from #7 to #1 (reverse order of serial dilution), positive control or samples. Cover with plate sealer and incubate at room temperature for 2 hours on microplate shaker (250 rpm).
4. Aspirate wells and wash 4 times with 300 µl of 1x Wash Buffer. Blot plate on absorbent paper

to remove any residual buffer.

5. Add 100 µl per well of 1x Detection Antibody-HRP conjugate working solution. Cover with plate sealer and incubate at room temperature for 90 minutes on microplate shaker (250 rpm). **Protect from light.**
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of Substrate Solution to each well. Incubate for 25-30 minutes on microplate shaker at room temperature. **Protect from light**.
8. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well using a microplate reader set to 450 nm within 2 min.

CALCULATION OF RESULTS

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log curve fit to more accurately quantify the standard dilutions.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

SPECIFICITY

PROTEINS	CROSS-REACTIVITY
Human Soluble DLL4 (HEK293)	100%
Mouse Soluble DLL4 (HEK293)	0
Human DLL1 (HEK293)	0
Human DKK1 (HEK293)	0

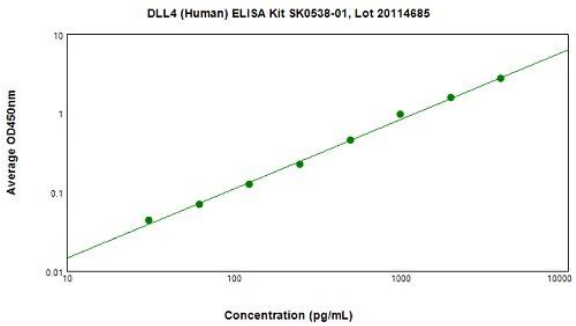
TYPICAL DATA

This standard curve data is provided for demonstration only. A new standard curve should be generated for each set of samples assayed.

STANDARD (PG/ML)	AVERAGE OD450NM (CORRECTED)
Blank	0 (0.061)
31.25	0.044
62.5	0.069
125	0.123
250	0.224
500	0.446
1000	0.959
2000	1.570
4000 (optional)	2.699

- Lot No.: 20114685
- Positive control: 250 ~ 1000 pg/mL (log-log)

Standard curve fit by log-log



SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard dilutions, samples or positive control to the well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl per well 1x Detection Antibody-HRP working solution to each well. Incubate 90 minutes on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl TMB Substrate Solution to each well. Incubate 25-30 min on the plate shaker at RT. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read at 450nm within 2 min.

Research Sample Determination

The research samples were diluted by Dilution Buffer DB10. Its linearity and recovery was assayed by Human Soluble DLL4 ELISA Kit SK00538-01

sample	Dilution Factor	Assayed (pg/mL)	Final (pg/mL)	Recovery (%)
Human EDTA Plasma	1 X	131.14	131.14	100
Human EDTA Plasma	2 X	63.462	126.924	97
Human EDTA Plasma	4 X	31.049	124.196	95
Human Serum	1 X	125.218	125.218	100
Human Serum	2 X	61.734	123.468	99
Human Serum	4 X	30.619	122.476	98