HUMAN ANGIOPOIETIN LIKE 4 (ANGPTL4) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN ANGPTL4 CONCENTRATIONS IN CELL CULTURE SUPERNATES, SERUM AND PLASMA

THIS PROTOCOL OR DATA IS PROVIDED FOR DEMONSTRATION ONLY.
ALWAYS REFER TO LOT SPECIFIC PROTCOL 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 ANGPTL4 ELISA**
Catalog No.	SK00309-01
Lot No.	
Formulation	96 T
Standard range	0.625 - 40 ng/mL
Sensitivity	0.3 ng/mL
Sample Volume	100 µl
Sample Type	Serum, EDTA Plasma, Cell Culture Supernates
Dilution Factors	4-fold dilution for serum or plasma samples **(Optimal dilutions should be determined by each laboratory for each application.)**
Specificity	Human ANGPTL4 only
Calibration	Human ANGPTL4 Full Length Recombinant
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 10%
Storage	2 – 8° C for 1 month. More information see page 2
This kit contains sufficient materials to run approximately 35 samples duplicated provided that assay is run according to protocol.	

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DESCRIPTION

This Human ANGPTL4 ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human ANGPTL4 from cell culture supernates, serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant full length human ANGPTL4 and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural ANGPTL4 samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for human ANGPTL4. The capture antibody can bind to the human ANGPTL4 in the standard and samples. After washing the plate of any unbound substances, a biotinyated antibody against human ANGPTL4 is added to the wells. After another washing of the plate, Streptavidin-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution (TMB) is added to the wells and color develops in direct proportion to the amount of human ANGPTL4 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
ANGPTL4 Microplate - 96 well polystyrene microplate coated with a purified antibody against human ANGPTL4.	**309-01-01**	**1 plate**
ANGPTL4 Standard – refer to lot of recombinant full length human ANGPTL4 in a buffered protein base with preservative; lyophilized.	**309-01-02**	**1 vial**
Detection Antibody Concentrate refer to lot, 10-fold concentrate of biotinylated antibody against ANGPTL4 with preservative; lyophilized.	**309-01-03**	**1 vial**
Positive Control - one vial of recombinant human ANGPTL4; lyophilized.	**309-01-04**	**1 vial**
Streptavidin-HRP Conjugate - 120 µL/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP.	**SAHRP**	**1 vial**
Dilution Buffer – 50 mL of buffered protein based solution with preservative.	**DB01**	**1 bottle**
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution - 11 mL of TMB substrate solution.	**TMB01**	**1 bottle**
Stop Solution - 11 mL of 0.5M HCl.	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1**
Plastic Pouch	**P01**	**1**

STORAGE

Unopened Kit: Store at 2 - 8° C for up to 1 month. For longer storage for up to 10 months, unopened Standard, Positive Control, Detection Antibody Concentrate and Dilution Buffer should be stored at -20° C. Streptavidin-HRP Conjugate 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 (250 – 300 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.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Serum and plasma samples require a 4-fold dilution. A suggested 4-fold dilution is 60 µL sample + 180 µL Dilution Buffer. **Optimal dilutions should be determined by each laboratory for each application.**

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 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of 1x Wash Buffer.

ANGPTL4 Standard - Reconstitute the ANGPTL4 standard with refer to lot of Dilution Buffer. This reconstitution produces a stock solution of 40 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of Dilution Buffer into tubes #1 to #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **40 ng/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 ng/mL).

Tube	Standard	Dilution Buffer	Concentration
stock	Powder	Refer to lot	40 ng/ml
# 1	250 µl of stock	250 µl	20 ng/ml
# 2	250 µl of 1	250 µl	10 ng/ml
# 3	250 µl of 2	250 µl	5 ng/ml
# 4	250 µl of 3	250 µl	2.5 ng/ml
# 5	250 µl of 4	250 µl	1.25 ng/ml
# 6	250 µl of 5	250 µl	0.625 ng/ml

Positive Control - Reconstitute the Positive Control with refer to lot of Dilution Buffer.

Detection Antibody - Reconstitute the Detection Antibody Concentrate with refer to lot of Dilution Buffer to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Pipette 11.88 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 120 µL of 100-fold concentrated stock solution to prepare working solution ***(protect from light)***.

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 of **Standard dilutions**, **samples**, or **positive control** per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with 1x Wash Buffer (300 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 100 µL of **Detection Antibody working solution** to each well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of **Streptavidin-HRP Conjugate working solution** to each well. Incubate for 60 minutes on microplate shaker at room temperature. **Protect from light.**
8. Repeat the aspiration/wash as in step 4.
9. Add 100 µL of **Substrate Solution** to each well. Incubate for refer to lot on microplate shaker at room temperature. **Protect from light.**
10. 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.
11. Determine the optical density of each well using a microplate reader set to 450 nm.

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.

TYPICAL DATA

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

Standard (ng/mL)	Average OD450 (Corrected)
Blank	0 (refer to lot)
0.625	0.037
1.25	0.065
2.5	0.104
5	0.258
10	0.430
20	0.913
40	1.715

SPECIFICITY

Proteins	Cross-reactivity (%)
Human ANGPTL4 full length	100
Human ANGPTL8/Betatrophin	0
Human Angiopoietin-3	0
Human ANGPTL3	0
Human Angiopoietin-1	0
Human Angiopoietin-2	0
Human Angiopoietin-4	0
Mouse Angiopoietin-3	0
Mouse ANGPTL3	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
⬇
Add 100 µl of standard dilutions, samples, or positive control to each well. Incubate 2 hours on the plate shaker at RT.
⬇
Aspirate and wash 4 times.
⬇
Add 100 µl Detection Antibody working solution to each well. Incubate 2 hours on the plate shaker at RT.
⬇
Aspirate and wash 4 times.
⬇
Add 100 µl Streptavidin-HRP conjugate working solution to each well. Incubate 60 min on the plate shaker at RT. **Protect from light.**
⬇
Aspirate and wash 4 times.
⬇
Add 100 µl Substrate Solution to each well. Incubate refer to lot on the plate shaker at RT. **Protect from light.**
⬇
Add 100 µl Stop Solution to each well. Read at 450nm.