

HUMAN ANGIOPOIETIN-LIKE 3 (ANGPTL3) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF
HUMAN ANGPTL3 CONCENTRATIONS IN CELL
CULTURE SUPERNATES, SERUM, AND PLASMA



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

PURCHASE INFORMATION:

ELISA Name	Human ANGPTL3 ELISA
Catalog No.	SK00311-01
Lot No.	
Formulation	96 T
Standard range	31.25-2000 pg/mL
Sensitivity	7.8 pg/mL
Sample Volume	100 µl
Dilution Factor	200 (Optimal dilutions should be determined by each laboratory for each application)
Sample Type	Serum, EDTA Plasma, Cell Culture Supernates
Specificity	Human ANGPTL3 only
Intra-assay Precision	4-8%
Inter-assay Precision	8-12%
Storage	2-8°C

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INTRODUCTION

Human ANGPTL3 immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure human ANGPTL3 in cell culture supernates, serum, and plasma. It contains recombinant human ANGPTL3 and antibodies raised against this protein. It has been shown to accurately quantify recombinant human ANGPTL3. Results obtained with naturally occurring ANGPTL3 samples showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that the immunoassay kit can be used to determine relative mass values for natural human ANGPTL3.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for ANGPTL3 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any ANGPTL3 present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated polyclonal antibody specific for ANGPTL3 is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, HRP link Streptavidin is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of ANGPTL3 bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

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_ The kit should not be used beyond the expiration date on the kit label.

_ Do not mix or substitute 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.

_ If samples generate values higher than the highest standard, dilute the samples with DILUTION BUFFER and repeat the assay.

_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors

have been tested in the immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
ANGPTL3 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against ANGPTL3.	311-01-01	1 plate
ANGPTL3 Standard – 2000 pg/vial of recombinant human ANGPTL3 in a buffered protein base with preservatives; lyophilized.	311-01-02	1 vial
Detection Antibody Concentrate – 1.05 mL/vial, 10-fold concentrated of biotinylated polyclonal antibody against ANGPTL3 with preservatives; lyophilized.	311-01-03	1 vial
Positive Control – one vial of recombinant ANGPTL3, lyophilized	311-01-04	1 vial
Streptavidin-HRP Conjugate - 120 µL/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP with preservatives	SAHRP	1 vial
Dilution Buffer - 60mL of buffered protein based solution with preservatives	DB01	2 bottles
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 piece
Plastic Pouch	P01	1 piece

STORAGE

Unopened Kit: Store at 2 - 8° C for up to 6 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate should be stored at -20°C or -70°C. Do not use kit past expiration date.

Opened / Reconstituted Reagents: Reconstituted

Standard and Detection Antibody Concentrate Solution SHOULD BE STORED at -20°C or -70°C for up to one month. Streptavidin-HRP Conjugate 100-fold concentrate (**protect from light**) and other components may be stored at 2 - 8°C for up to 6 months.

Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack, reseal along entire edge of zip-seal. Microplate may be stored for up to 6 months at 2 - 8°C after opening.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

PRECAUTIONS FOR USE

All reagents should be considered as potentially hazardous. The stop solution contains diluted hydrochloric acid. Appropriate care, therefore, should be taken while handling this solution. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.

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 1000 x 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 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store

samples at ≤-20° C. Avoid repeated freeze-thaw cycles.

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

SAMPLE PREPARATION

Serum and plasma samples may need a 200-fold dilution. A suggested 200-fold dilution is 5 µL sample + 995 µL Dilution Buffer.

Optimal dilutions should be determined by each laboratory for each application.

Use polypropylene test tubes.

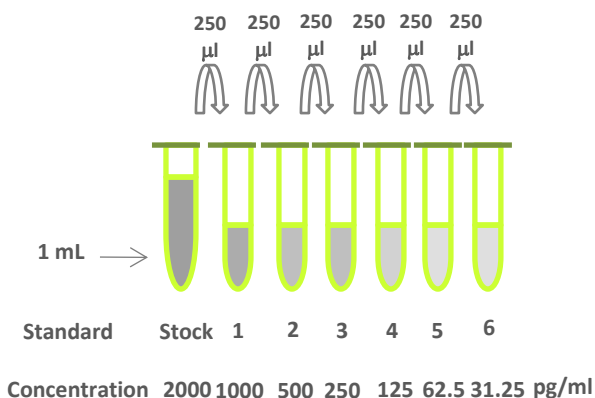
REAGENT PREPARATION

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 Wash Buffer.

ANGPTL3 Standard - Refer to vial label for reconstitution volume. Reconstitute the **ANGPTL3** standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 2000 pg/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 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	1.0 ml	2000 pg/ml
# 1	250µl of stock	250µl	1000 pg/ml
# 2	250µl of 1	250µl	500 pg/ml
# 3	250µl of 2	250µl	250 pg/ml
# 4	250µl of 3	250µl	125 pg/ml
# 5	250µl of 4	250µl	62.5 pg/ml
# 6	250µl of 5	250µl	31.25 pg/ml



Detection Antibody - Reconstitute the **Detection Antibody Concentrate** with 1.05mL 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 1.05mL of 10-fold concentrated stock solution to prepare working solution.

Streptavidin HRP Conjugate - Transfer 120 µL of 100-fold concentrated stock solution to 11.88 mL of Dilution Buffer to prepare working solution. **Note:** 1x working solution of Streptavidin-HRP Conjugate should be used within a few days (**protect from light**).

Positive Control - Reconstitute the positive control with 1.0 mL of Dilution Buffer to make positive control working solution. **Note:** Positive control working solution should be used within a few days (store at -20°C or -70°C).

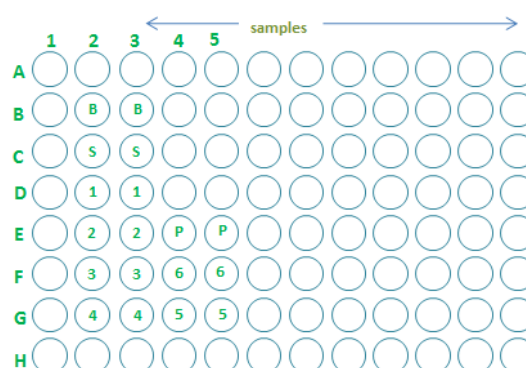
ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that blank, standards, positive control and samples be assayed in duplicates.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the plastic pouch with the desiccant pack, reseal.
3. Add 100 µL of Dilution Buffer to Blank wells (B2, B3).
4. Add 100 µL of Standard (C2, C3 to G2, G3 and F4, F5 to G4, G5), sample, or positive control (E4, E5) per well. Cover with plate sealer. Incubate for 2

hours on micro-plate shaker at room temperature. A plate layout is provided to record standards and samples assayed.

5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with 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.
6. Add 100 µL of Detection Antibody working solution to each well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Streptavidin-HRP Conjugate working solution to each well. Incubate for 60 minutes on micro-plate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of Substrate Solution to each well. Incubate for 3-6 minutes on micro-plate shaker at room temperature. **Protect from light.**
11. 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.
12. Determine the optical density of each well within 15 minutes, using a micro-plate reader set to 450 nm.



CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control, and sample and subtract the

average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a log-log curve fit. As an alternative, 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. The data may be linearized by plotting the log of the ANGPTL3 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

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.

ANGPTL3 (PG/ML)	CORRECTED (450NM)
Blank	0 (0.108)
31.25	0.051
62.5	0.071
125	0.128
250	0.234
500	0.477
1000	0.936
2000	1.860

- Lot No.:
- Positive Control :

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant human ANGPTL3.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of ANGPTL3 was 7.8 pg/mL.

SPECIFICITY

PROTEINS	CROSSREACTIVITY (%)
Human ANGPTL 3	100%
Mouse ANGPTL 3	0
Human ANGPTL 4	0
Mouse ANGPTL 4	0
Human Angiopoietin-1	0
Human Angiopoietin-2	0
Human Angiopoietin-4	0
Intergrin $\alpha V\beta 3$	0

LINEARITY

To assess the linearity of the assay, pooled research human serum samples were diluted with Dilution Buffer and assayed.

DILUTION FACTOR	ASSAYED (PG/ML)	FINAL (NG/ML)	RECOVERY (%)
200 X	1731.771	346.3542	100
400 X	777.180	310.872	89.8

To assess the linearity of the assay, pooled research human EDTA plasma samples were diluted with Dilution Buffer and assayed.

DILUTION FACTOR	ASSAYED (PG/ML)	FINAL (NG/ML)	RECOVERY (%)
200 X	3474.379	694.8758	100
400 X	1680.314	672.1256	96.7

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS

↓
Add 100 µl of standards, samples, positive control to each well. Incubate for 2 hours on the plate shaker at RT.

↓
Aspirate and wash 4 times.

↓
Add 100 µl Detection Antibody working solution to each well. Incubate for 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 3-6 min on the plate shaker. **Protect from light.**

↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min

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