Quantikine® ELISA

Human Angiopoietin-like 3 Immunoassay

Catalog Number DANL30

For the quantitative determination of human Angiopoietin-like 3 (ANGPTL3) concentrations in cell culture supernates, serum, and plasma.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Human Angiopoietin-like 3 (ANGPTL3), also known as angiopoietin-5, is a secreted glycoprotein that is structurally related to the angiopoietins. Mature human ANGPTL3 contains an N-terminal coiled-coil domain, a C-terminal fibrinogen-like domain, and a linker region. Unlike other angiopoietin-like proteins, ANGPTL3 is almost exclusively expressed in the liver (1-4).

ANGPTL3 plays an important role in lipid metabolism. In humans, genome-wide association studies have shown that sequence variants in the locus containing the ANGPTL3 gene are associated with different plasma triglyceride levels (5, 6). Population-based gene sequence analysis also reveals that a loss of function mutation in the ANGPTL3 gene is commonly present in those individuals with low plasma triglyceride levels (7). ANGPTL3 can directly inhibit lipoprotein lipase (LPL), the enzyme responsible for hydrolyzing circulating triglycerides (8). In mice, abolishing ANGPTL3 gene expression leads to elevated LPL activity and hypolipidemia. When the hyperlipidemic mice are treated with a monoclonal antibody that interferes with the binding of ANGPTL3 to LPL, their serum triglyceride levels are significantly reduced (9, 10). The functional domain that mediates the interaction between ANGPTL3 and LPL has been mapped to the N-terminal region, termed specific epitope 1 (11). The linker region in ANGPTL3 is susceptible to protease cleavage. When the cleavage occurs between amino acid 221 and 224, it separates the protein into an N-terminal coiled-coil domain- containing fragment and a C-terminal fibrinogen-like domain-containing fragment. Compared to the full-length protein, the N-terminal fragment has increased bioactivity for lowering serum triglyceride (12). ANGPTL3 is also able to inhibit endothelial lipase, whose preferred substrate is high density lipoprotein (HDL). As such, ANGPTL3 may modulate serum HDL levels (13, 14). In addition to lipid metabolism, ANGPTL3 is also involved in angiogenesis. The fibrinogen-like domain of ANGPTL3 can interact with integrin αVβ3 to induce endothelial cell adhesion, migration, and neovascularization (15). Furthermore, ANGPTL3 may promote the expansion of hematopoietic stem cells (16). Both the full-length ANGPTL3 and its cleaved fragments are detectable in the circulation (12). It has been reported that ANGPTL3 serum levels are different in patients with certain pathological conditions when compared to those in normal individuals. For instance, it is elevated in familial hypercholesterolemia, metabolic syndrome, and insulin resistance. In hemodialysis, however, it is reduced (17-20).

The Quantikine® Human Angiopoietin-like 3 Immunoassay is a 3.5 hour solid-phase ELISA designed to measure human ANGPTL3 in cell culture supernates, serum, and plasma. It contains *Sf* 21-expressed recombinant human ANGPTL3 and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human ANGPTL3 showed linear curves that were parallel to the standard curves obtained using the Quantikine® kit standards. These results indicate that this 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 human 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, an enzyme-linked polyclonal antibody specific for human ANGPTL3 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of ANGPTL3 bound. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- 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.
- If samples generate values higher than the highest standard, further dilute the samples with calibrator diluent and repeat the assay.
- Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine® Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human ANGPTL3 Microplate	893734	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human ANGPTL3.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Human ANGPTL3 Standard	893736	Recombinant human ANGPTL3 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Aliquot and store at ≤ -20 °C for up to 1 month.* Avoid repeated freeze-thaw cycles.
Human ANGPTL3 Conjugate	893735	21 mL of a polyclonal antibody specific for human ANGPTL3 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-76	895812	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD6Q	895128	21 mL of animal serum with preservatives. *Use diluted 1:5 in this assay.*	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	6 mL of 2 N sulfuric acid.	
Plate Sealers	N/A	4 adhesive strips.	

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- **Polypropylene** test tubes for dilution of standards and samples.
- Human ANGPTL3 Controls (optional; R&D Systems®, Catalog # QC44).

PRECAUTIONS

Calibrator Diluent RD6Q contains sodium azide which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the MSDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

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 at room temperature 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 or heparin 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: *Citrate plasma has not been validated for use in this assay.*

SAMPLE PREPARATION

Use polypropylene tubes.

Serum and plasma samples require a 50-fold dilution. A suggested 50-fold dilution is 20 µL of sample + 980 µL of Calibrator Diluent RD6Q (diluted 1:5).*

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

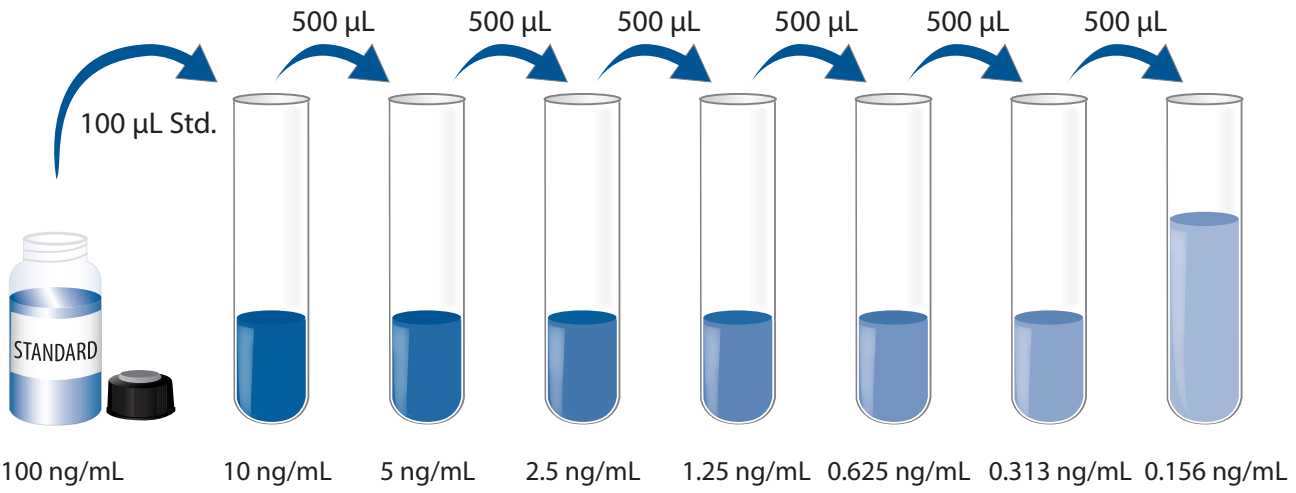
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Calibrator Diluent RD6Q (diluted 1:5) - Add 20 mL of Calibrator Diluent RD6Q to 80 mL of deionized or distilled water to prepare 100 mL of Calibrator Diluent RD6Q (diluted 1:5).

Human ANGPTL3 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human ANGPTL3 Standard with deionized or distilled water. This reconstitution produces a stock solution of 100 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Use polypropylene tubes. Pipette 900 µL of Calibrator Diluent RD6Q (diluted1:5) into the 10 ng/mL tube. Pipette 500 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 10 ng/mL standard serves as the high standard. Calibrator Diluent RD6Q (diluted 1:5) serves as the zero standard (0 ng/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, controls, and standards be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of Assay Diluent RD1-76 to each well.
4. Add 50 µL of standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.
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 (400 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 200 µL of Human ANGPTL3 Conjugate to each well. Cover with the adhesive strip provided. Incubate for 1 hour at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 50 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 the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Samples may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

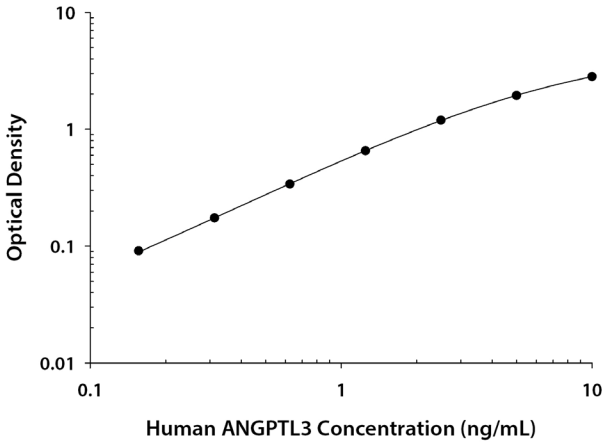
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) 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 human 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.



(ng/mL)	O.D.	Average	Corrected
0	0.015 0.016	0.016	—
0.156	0.106 0.107	0.107	0.091
0.313	0.187 0.192	0.190	0.174
0.625	0.354 0.358	0.356	0.340
1.25	0.646 0.691	0.669	0.653
2.5	1.205 1.210	1.208	1.192
5	1.940 1.977	1.959	1.943
10	2.820 2.846	2.833	2.817

PRECISION

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

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	20	20	20
Mean (ng/mL)	1.05	3.36	6.80	1.02	3.18	6.74
Standard deviation	0.04	0.09	0.18	0.07	0.20	0.60
CV (%)	3.8	2.7	2.6	6.9	6.3	8.9

RECOVERY

The recovery of human ANGPTL3 spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	103	99-107%
Serum* (n=4)	96	81-115%
EDTA plasma* (n=4)	102	85-120%
Heparin plasma* (n=4)	96	85-109%

*Samples were diluted prior to assay as directed in the Sample Preparation section.

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human ANGPTL3 were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=4)	Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)
1:2	Average % of Expected	100	103	102	104
	Range (%)	95-109	100-104	99-107	102-107
1:4	Average % of Expected	97	108	105	106
	Range (%)	96-99	107-110	99-108	101-111
1:8	Average % of Expected	97	109	110	110
	Range (%)	93-99	108-110	105-114	107-112
1:16	Average % of Expected	95	109	110	109
	Range (%)	92-98	103-114	108-112	104-114

*Samples were diluted prior to assay as directed in the Sample Preparation section.

SENSITIVITY

Twenty assays were evaluated and the minimum detectable dose (MDD) of human ANGPTL3 ranged from 0.002-0.035 ng/mL. The mean MDD was 0.011 ng/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified *Sf* 21-expressed recombinant human Angiopoietin-like 3 produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human ANGPTL3 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=36)	94.8	46.1-157	29.8
EDTA plasma (n=36)	108	55.8-172	29.7
Heparin plasma (n=36)	94.1	47.7-194	28.7

Cell Culture Supernates - HepG2 human hepatocellular carcinoma cells were cultured in DMEM supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate until confluent. An aliquot of the cell culture supernate was removed, assayed for human ANGPTL3, and measured 2.62 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant human ANGPTL3.

The factors listed below were prepared at 100 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 100 ng/mL in a mid-range recombinant human ANGPTL3 control were also assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

Angiopoietin-1
Angiopoietin-2
Angiopoietin-4
Angiopoietin-like 1
Angiopoietin-like 2
Angiopoietin-like 4
Angiopoietin-like 7

Recombinant mouse:

Angiopoietin-3
Angiopoietin-like 3
Angiopoietin-like 4
Angiopoietin-like 7

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