ANGPTL3 (human) Serum ELISA Kit

(Catalog #K4914-100; 100 assays; Store kit at 4° C)

I. Description:

The angiopoietins are a family of growth factors that are specific for vascular endothelium. The full-length cDNA encoding angiopoietin-like protein 3 (ANGPTL3) from a human fetal liver/spleen cDNA library has 460-amino acid and the characteristic structure of angiopoietins: a signal peptide, an extended helical domain predicted to form dimeric or trimeric coiled-coils, a short linker peptide, and a globular fibrinogen-like domain (FLD). Human ANGPTL3 shares 76% amino acid sequence identity with mouse Angptl3. Northern blot analysis of human tissues showed a preferential expression of 4 ANGPTL3 transcripts being 4.5, 3.0, 2.8, and 1.7 kb in liver. ANGPTL3 can induce angiogenesis in the rat corneal assay. The FLD alone was sufficient to induce endothelial cell adhesion and in vivo angiogenesis. Microarray analysis showed that mouse hematopoietic stem cell (HSC)-supportive fetal liver CD3-positive cells expressed Angptl2 and Angptl3. The ANGPTL3 ELISA Kit is to be used for the *in vitro* quantitative determination of human ANGPTL3 in biological fluids. This assay is a sandwich Enzyme Linked-Immunosorbent Assay (ELISA) for quantitative determination of human ANGPTL3 in biological fluids. A monoclonal antibody specific for ANGPTL3 has been precoated onto the 96-well microtiter plate. Standards and samples are pipetted into the wells for binding to the coated antibody. After extensive washing to remove unbound compounds, ANGPTL3 is recognized by the addition of a purified polyclonal antibody specific for ANGPTL3 (Detection Antibody). After removal of excess polyclonal antibody, HRP conjugated anti-rabbit IgG (Detector) is added. Following a final washing, peroxidase activity is quantified using the substrate 3,3',5,5'-tetramethylbenzidine (TMB). The intensity of the color reaction is measured at 450 nm after acidification and is directly proportional to the concentration of ANGPTL3 in the samples.
The assay range is 0.156–10 ng/ml ANGPTL3/ml. The lowest level of ANGPTL3 that can be detected by this assay is 75 pg/ml.

II. Kit Contents:

Component	100 Assays	Part Number
1 plate coated with human ANGPTL3 Antibody	(6 x 16-well strips)	K4914-100-1
2 bottle Wash Buffer 10X	2 x 30 ml	K4914-100-2
2 bottles ELISA Buffer 10X	2 x 30 ml	K4914-100-3
1 vial Detection Antibody	50 µl	K4914-100-4
1 vial HRP 100X (HRP Conjugated anti-rabbit IgG)	150 µl	K4914-100-5
1 vial human ANGPTL3 Standard (lyophilized)	20 ng	K4914-100-6
1 bottle TMB Substrate Solution	12 ml	K4914-100-7
1 bottle Stop Solution	12 ml	K4914-100-8
2 plate sealers (plastic film)	2	K4914-100-9
2 silica Gel Minibags	2	K4914-100-10

III. Storage Conditions

Reagents must be stored at 2 - 8°C when not in use. Bring reagents to room temperature before use. Do not expose reagents to temperatures greater than 25°C.

IV. Assay Procedure (Read the ENTIRE Protocol Before Proceeding)

1. Test Samples/Standards/QC Sample: (We recommend these be run in duplicate)

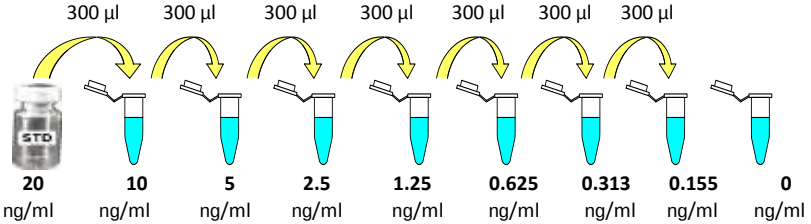
a) **Serum**: Use a serum separator tube. Let samples clot at room temperature for 30 min before centrifugation for 20 min at 1000 x g. Assay freshly prepared serum or store serum in aliquots at -20°C for future use. Avoid repeated freeze/thaw cycles.

b) **Plasma:** Collect plasma using heparin or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay freshly prepared plasma or store plasma sample in aliquot at ≤ -20°C for later use. Avoid repeated freeze/ thaw cycles.Note: **Serum, Plasma, Urine** or **Cell Culture Supernatant** has to be diluted in ELISA Buffer 1X. Samples containing visible precipitates must be clarified before use. As starting point 1/50 dilution of serum or plasma are recommended.

c) **Standards**: Reconstitute human ANGPTL3 Standard with 1 ml of dH_2O to produce a stock solution (20 ng/ml). Mix the Stock solution to ensure complete reconstitution. Allow to sit for a minimum of 15 min. The reconstituted standard should be aliquoted and stored at -20°C.

d) Prepare Standard Curve using 2-fold serial dilutions with 1X ELISA Buffer:

To obtain	Add	Into
10 ng/ml	300 µl of ANGPTL3 (20 ng/ml)	300 µl of 1X ELISA Buffer
5 ng/ml	300 µl of ANGPTL3 (10 ng/ml)	300 µl of 1X ELISA Buffer
2.5 ng/ml	300 µl of ANGPTL3 (5 ng/ml)	300 µl of 1X ELISA Buffer
1.25 ng/ml	300 µl of ANGPTL3 (2.5 ng/ml)	300 µl of 1X ELISA Buffer
0.625 ng/ml	300 µl of ANGPTL3 (1.25 ng/ml)	300 µl of 1X ELISA Buffer
0.313 ng/ml	300 µl of ANGPTL3 (0.625 ng/ml)	300 µl of 1X ELISA Buffer
0.156 ng/ml	300 µl of ANGPTL3 (0.313 ng/ml)	300 µl of 1X ELISA Buffer
0 ng/ml	300 µl of 1X ELISA Buffer	Empty tube



2. Reagent Preparation: (Prepare just the appropriate amounts for the assay)

a) Wash Buffer 10X has to be diluted with deionized water 1:10 before use (e.g. 50 ml Wash Buffer 10X + 450 ml water) to obtain Wash Buffer 1X.

b) **ELISA Buffer 10X** has to be diluted with deionized water 1:10 before use (e.g. 20 ml ELISA Buffer 10X + 180 ml water) to obtain ELISA Buffer 1X.

c) Detection Antibody (DET) has to be diluted to 1:250 in ELISA Buffer 1X (40 µl DET + 10 ml ELISA Buffer 1X).

d) **HRP 100X (HRP Conjugated anti-rabbit IgG)** has to be diluted to the working concentration by adding 100 µl in 10 ml of ELISA Buffer 1X (1:100). Note: The diluted HRP must be used within 1 hr of preparation.

3. Assay Protocol:

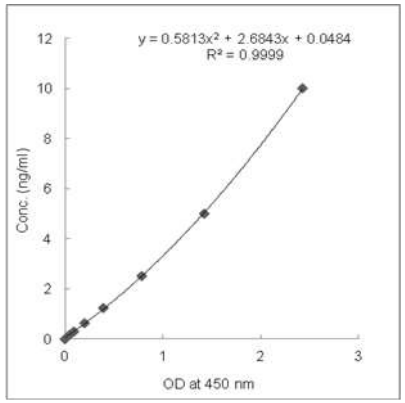
1. Determine the number of 16-well strips needed for assay and insert them into the frame for current use. The extra strips should be resealed in the foil pouch and can be stored at 4°C for up to 1 month.
2. Add 100 µl of the **Standards** and **Samples** into the appropriate wells in duplicate.
3. Cover plate with plate sealer and incubate for 1 hr at 37°C.
4. Aspirate and wash x 3 with 300 µl of 1X Wash Buffer. Remove liquid completely after the last wash.
5. Add 100 µl to each well of the **Detection Antibody (DET).**
6. Cover plate with plate sealer and incubate for 1 hr at 37°C.
7. Aspirate and wash x 3 with 300 µl of **1X Wash Buffer**.
8. Add 100 µl of the 1X diluted HRP.
9. Cover plate with plate sealer and incubate for 1 hr at 37°C.
10. Aspirate and wash x 5 with 300 µl of 1X Wash Buffer. Remove liquid completely after the last wash.
11. Add 100 µl **TMB Substrate Solution** to each well.
12. Allow the color to develop at room temperature in the dark for 10 min.
13. Stop the reaction by adding 100 µl of **Stop Solution to** each well.
14. Measure the OD at 450 nm in an ELISA plate reader within 30 min.

V. Calculations:

- Average the duplicate readings for each standard, and sample and subtract the average blank value (obtained with the 0 ng/ml point).
- Generate a Standard Curve by plotting the average absorbance on the horizontal (X) axis vs. the corresponding concentration (ng /ml) on the vertical (Y) axis. (See Typical Data below)
- Calculate the concentration of test samples by interpolation of the standard curve regression curve as shown below in the form of a quadratic equation.
- If the Test Samples were diluted, multiply the interpolated values by the dilution factor to calculate the corrected human ANGPTL3 concentrations.

VI. Performance Characteristics:

The following data are obtained using the different concentrations of standard as described in this protocol.



Standard hANGPTL3 (ng/ml)	Optical Density (mean)
10	2.428
5	1.418
2.5	0.787
1.25	0.391
0.625	0.198
0.313	0.088
0.156	0.049
0	0

1. **Intra-assay precision:** Six samples of known concentrations of human ANGPTL3 were assayed in replicates 6 times to test precision within an assay.

Samples	Mean	SD	CV (%)	n
1	81.69	0.96	1.17	6
2	111.58	2.59	2.32	6
3	108.06	1.07	0.99	6
4	145.16	2.19	1.51	6
5	130.98	3.00	2.29	6
6	76.31	1.18	1.55	6

2. **Inter-assay precision:** Six samples of known concentrations of human ANGPTL3 were assayed in 6 separate assays to test precision between assays.

Samples	Mean	SD	CV (%)	n
1	81.54	3.49	4.29	6
2	109.32	6.52	5.96	6
3	145.20	9.61	6.62	6
4	129.35	10.75	8.31	6
5	126.42	10.54	8.33	6
6	75.04	5.82	7.75	6

3. **Recovery:** When samples (serum) are spiked with known concentrations of human ANGPTL3, the recovery averages 89% (range from 80 to 105%).

Samples	Average Recovery (%)	Range (%)
1	86.86	80-100
2	90.01	85-105
3	89.43	85-105

VII. Troubleshooting:

PROBLEM	POSSIBLE CAUSES	SOLUTIONS
No signal or weak signal	Omission of key reagent	Check that all reagents have been added in the correct order.
	Washes too stringent	Use an automated plate washer if possible.
	Incubation times inadequate	Incubation times should be followed as indicated in the manual.
	Plate reader settings not optimal	Verify the wavelength and filter setting in the plate reader.
	Incorrect assay temperature	Use recommended incubation temperature. Bring substrates to room temperature before use.
High background	Concentration of detector too high	Use recommended dilution factor.
	Inadequate washing	Ensure all wells are filling wash buffer and are aspirated completely.
Poor standard curve	Wells not completely aspirated	Completely aspirate wells between steps.
	Reagents poorly mixed	Be sure that reagents are thoroughly mixed.
Unexpected results	Omission of reagents	Be sure that reagents were prepared correctly and added in the correct order.
	Dilution error	Check pipetting technique and double-check calculations.

VIII. Related Products:

- ANGPTL3 (mouse/rat) Serum ELISA Kit (Cat. No. K4915-100)
- ANGPTL6 (human) Serum ELISA Kit (Cat. No. K4916-100)
- ANGPTL1 Antibody (NT) (Cat. No. 6761-100)
- ANGPT2 Antibody (CT) (Cat. No. 6759-100)
- ANGPT1 Antibody (CT) (Cat. No. 6760-100)

FOR RESEARCH USE ONLY! Not to be used on humans.