Quantikine® ELISA

Mouse Angiopoietin-like 3 Immunoassay

Catalog Number MANL30

For the quantitative determination of mouse Angiopoietin-like 3 (ANGPT-L3) concentrations in cell culture supernates, serum, and plasma.

Note: The standard reconstitution method has changed. Read this package insert in its entirety before using this product.

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

TABLE OF CONTENTS

MANUFACTURED AND DISTRIBUTED BY:

USA & Canada | R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413, USA
TEL: (800) 343-7475 (612) 379-2956 FAX: (612) 656-4400
E-MAIL: info@RnDSystems.com

DISTRIBUTED BY:

UK & Europe | R&D Systems Europe, Ltd.
19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420
E-MAIL: info@RnDSystems.co.uk

China | R&D Systems China Co., Ltd.
24A1 Hua Min Empire Plaza, 726 West Yan An Road, Shanghai PRC 200050
TEL: +86 (21) 52380373 FAX: +86 (21) 52371001
E-MAIL: info@RnDSystemsChina.com.cn

INTRODUCTION

Angiopoietin-like 3 (ANGPT-L3) is a secreted glycoprotein that plays an important role in fatty acid metabolism. It is one of several molecules with structural similarity to the angiopoietins, which also contain an N-terminal coiled coil domain and a C-terminal fibrinogen-like domain (1-4). Mouse ANGPT-L3 shares 22%-30% amino acid (aa) sequence identity with mouse ANGPT-L1, 2, 4, 6, and 7. It shares 77% aa sequence identity with human ANGPT-L3. ANGPT-L3 is expressed in the liver from early in development through adulthood (5, 6). It is found as 70 kDa, 50 kDa, and 32 kDa forms and can form weakly associated non-covalent multimers *in vitro* (6). ANGPT-L3 directly inhibits lipoprotein lipase (LPL) and endothelial lipase, enzymes responsible for hydrolyzing circulating triglycerides and HDL phospholipids (7, 8). This activity requires a putative heparin-binding motif which is N-terminal to the coiled coil domain (9).

ANGPT-L3 is proteolytically cleaved in the liver by proprotein convertases (10). Full length ANGPT-L3 circulates in the plasma as do the separated N- and C-terminal fragments which contain the coiled coil domain and fibrinogen-like domains, respectively (9). Cleavage serves to activate ANGPT-L3, as the released N-terminal fragment is more potent than full length ANGPT-L3 at increasing plasma triglycerides and inhibiting endothelial lipase (9, 10). ANGPT-L3 does not bind the angiopoietin receptors Tie-1 or Tie-2, but its fibrinogen-like domain interacts with integrin αVβ3 to induce endothelial cell adhesion, migration, and neovascularization (11). ANGPT-L3 also promotes the expansion of hematopoietic stem cells (12).

ANGPT-L3 promotes an increase in circulating triglyceride levels but does not alter VLDL or HDL secretion or uptake (7, 9, 13). ANGPT-L3 knockout mice are hypolipidemic and have elevated LPL activity (14). ANGPT-L3 expression *in vivo* is upregulated by liver X receptor (LXR) agonists and downregulated by insulin, leptin, and agonists of thyroid hormone receptor beta (TRβ) or peroxisome proliferator-activated receptor beta (PPARβ) (15-18). Dysregulated ANGPT-L3 expression and elevated plasma triglyceride levels are characteristic of some strains of obese and diabetic mice (7, 13, 16). In humans, serum ANGPT-L3 levels are positively correlated with serum HDL-Cholesterol and adiponectin levels as well as with arterial intima-media thickness, an indicator for the progression of atherosclerosis (19, 20).

The Quantikine® Mouse Angiopoietin-like 3 Immunoassay is a 4.5 hour solid phase ELISA designed to measure mouse ANGPT-L3 in cell culture supernates, serum, and plasma. It contains *Sf* 21-expressed recombinant mouse ANGPT-L3 and has been shown to accurately quantitate the recombinant factor. Results obtained using natural mouse ANGPT-L3 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 mouse ANGPT-L3.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse ANGPT-L3 has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any ANGPT-L3 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse ANGPT-L3 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 ANGPT-L3 bound in the initial step. 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.
- For best results, pipette reagents and samples into the center of each well.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- 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.

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
Mouse ANGPT-L3 Microplate	893718	96 well polystyrene microplate (12 strips of 8 wells) coated with a rat monoclonal antibody specific for mouse ANGPT-L3.	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.*
Mouse ANGPT-L3 Standard	893720	2 vials of recombinant mouse ANGPT-L3 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Use a new standard and control for each assay. Discard after use.
Mouse ANGPT-L3 Control	893721	2 vials of recombinant mouse ANGPT-L3 in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label.	
Mouse ANGPT-L3 Conjugate	893719	12 mL of a polyclonal antibody specific for mouse ANGPT-L3 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-21	895215	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-26 Concentrate	895525	21 mL of a concentrated buffered protein base with preservatives. *Use diluted 1:4 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	895174	23 mL of diluted hydrochloric 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.

PRECAUTIONS

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. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000 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 20 minutes at 2000 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.*
Do not use icteric samples.

SAMPLE PREPARATION

Serum and plasma samples require a 100-fold dilution. A suggested 100-fold dilution is 10 µL of sample + 490 µL of Calibrator Diluent RD5-26 (diluted 1:4)* followed by 70 µL of the diluted sample + 70 µL of Calibrator Diluent RD5-26 (diluted 1:4)*.

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Mouse ANGPT-L3 Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

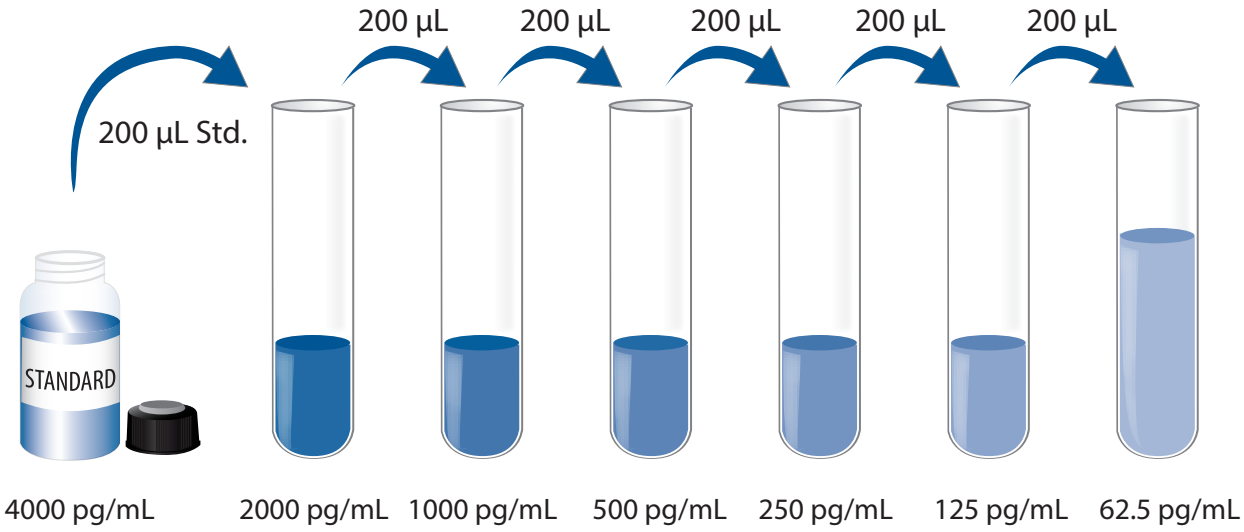
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Calibrator Diluent RD5-26 (diluted 1:4) - Add 20 mL of Calibrator Diluent RD5-26 Concentrate to 60 mL of deionized or distilled water to prepare 80 mL of Calibrator Diluent RD5-26 (diluted 1:4).

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 µL of the resultant mixture is required per well.

Mouse ANGPT-L3 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse ANGPT-L3 Standard with Calibrator Diluent RD5-26 (diluted 1:4). Do not substitute other diluents. This reconstitution produces a stock solution of 4000 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum 5 minutes with gentle agitation prior to making dilutions.

Use polypropylene tubes. Pipette 200 µL of Calibrator Diluent RD5-26 (diluted 1:4) into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Mouse ANGPT-L3 Standard (4000 pg/mL) serves as the high standard. Calibrator Diluent RD5-26 (diluted 1:4) serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

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

1. Prepare all reagents, working standards, control, 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, reseal.
3. Add 50 µL of Assay Diluent RD1-21 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 four times for a total of five 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 100 µL of Mouse ANGPT-L3 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 100 µL of Stop Solution to each well. 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

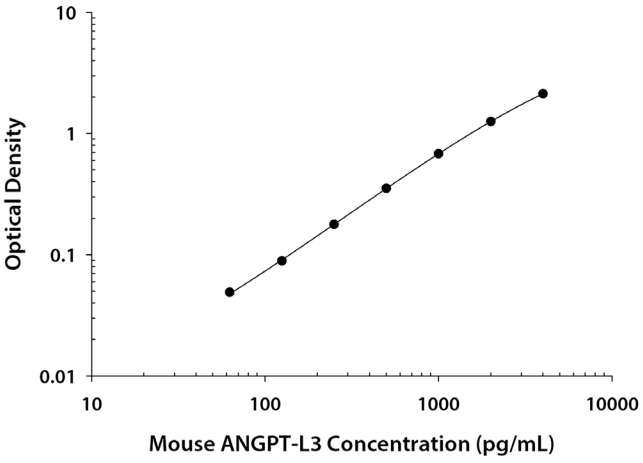
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 mouse ANGPT-L3 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.



(pg/mL)	O.D.	Average	Corrected
0	0.014 0.018	0.016	—
62.5	0.062 0.067	0.065	0.049
125	0.102 0.108	0.105	0.089
250	0.190 0.197	0.194	0.178
500	0.357 0.380	0.369	0.353
1000	0.691 0.702	0.697	0.681
2000	1.230 1.313	1.272	1.256
4000	2.067 2.220	2.144	2.128

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 forty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of kit components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	190	383	1346	197	418	1449
Standard deviation	10.9	23.6	79.1	17.4	27.1	90.5
CV (%)	5.7	6.2	5.9	8.8	6.5	6.2

RECOVERY

The recovery of mouse ANGPT-L3 spiked to levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture supernates (n=4)	103	93-120%

LINEARITY

To assess the linearity of the assay, samples containing high concentrations of mouse ANGPT-L3 were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture samples (n=2)	Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)
1:2	Average % of Expected	101	98	98	96
	Range (%)	100-101	96-99	93-102	91-99
1:4	Average % of Expected	103	98	98	95
	Range (%)	100-106	97-100	90-105	90-101
1:8	Average % of Expected	103	99	96	92
	Range (%)	97-110	96-102	88-103	87-99
1:16	Average % of Expected	91	97	96	92
	Range (%)	91-91	93-99	88-105	85-96

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

SENSITIVITY

Fifty two assays were evaluated and the minimum detectable dose (MDD) of mouse ANGPT-L3 ranged from 1.64-9.62 pg/mL. The mean MDD was 4.29 pg/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 mouse ANGPT-L3 produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Samples were evaluated for the presence of mouse ANGPT-L3 in this assay.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=20)	244	115-435	78.7
EDTA plasma (n=20)	311	137-486	89.6
Heparin plasma (n=20)	234	139-343	52.0

Cell Culture Supernates:

Mouse liver tissue from one mouse was cut into 1-2 mm pieces and cultured in 100 mL of RPMI supplemented with 10% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate for 2 days. Cells were unstimulated or stimulated with 1.0 mg/mL of lipopolysaccharide for 2 days. Aliquots of the cell culture supernates were removed and assayed for mouse ANGPT-L3.

Mouse heart tissue from two mice was cut into 1-2 mm pieces and cultured in 100 mL of RPMI supplemented with 10% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate for 1 day. Cells were unstimulated or stimulated with 1.0 mg/mL of lipopolysaccharide for 1 day. Aliquots of the cell culture supernates were removed and assayed for mouse ANGPT-L3.

Mouse lung tissue from three mice was cut into 1-2 mm pieces and cultured in 100 mL of RPMI supplemented with 10% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate for 1 day. An aliquot of the cell culture supernate was removed and assayed for mouse ANGPT-L3.

Tissue Type	Observed Levels (pg/mL)
Liver, unstimulated	918
Liver, stimulated	1045
Heart, unstimulated	152
Heart, stimulated	131
Lung	232

SPECIFICITY

This assay recognizes recombinant and natural mouse ANGPT-L3.

The factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range recombinant mouse ANGPT-L3 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:	Recombinant human:
Angiopoietin-3	Angiopoietin-like 3 (aa 17-460)
Angiopoietin-like 2	Angiopoietin-like 3 (aa 17-220)
Angiopoietin-like 4	LPL (aa 28-154)
Angiopoietin-like 7	

Rat serum was not detectable in this assay.

This assay detects full-length and 27 kDa N-terminal cleaved mouse ANGPT-L3 but does not detect the 38 kDa C-terminal cleavage fragment.

REFERENCES

1. Hato, T. *et al.* (2008) Trends Cardiovasc. Med. **18**:6.
2. Li, C. (2006) Curr. Opin. Lipidol. **17**:152.
3. Oike, Y. *et al.* (2004) Int. J. Hematol. **80**:21.
4. Kersten, S. (2005) Biochem. Soc. Transact. **33**:1059.
5. Conklin, D. *et al.* (1999) Genomics **62**:477.
6. Ge, H. *et al.* (2005) J. Lipid Res. **46**:1484.
7. Shimizugawa, T. *et al.* (2002) J. Biol. Chem. **277**:33742.
8. Shimamura, M. *et al.* (2007) Arterioscler. Thromb. Vasc. Biol. **27**:366.
9. Ono, M. *et al.* (2003) J. Biol. Chem. **278**:41804.
10. Jin, W. *et al.* (2007) Cell Metab. **6**:129.
11. Camenisch, G. *et al.* (2002) J. Biol. Chem. **277**:17281.
12. Zhang, C.C. *et al.* (2006) Nat. Med. **12**:240.
13. Koishi, R. *et al.* (2002) Nat. Genet. **30**:151.
14. Koster, A. *et al.* (2005) Endocrinology **146**:4943.
15. Inaba, T. *et al.* (2003) J. Biol. Chem. **278**:21344.
16. Shimamura, M. *et al.* (2004) Biochem. Biophys. Res. Commun. **322**:1080.
17. Fugier, C. *et al.* (2006) J. Biol. Chem. **281**:11553.
18. Matsusue, K. *et al.* (2006) Mol. Cell. Endocrinol. **256**:23.
19. Hatsuda, S. *et al.* (2006) J. Vasc. Res. **44**:61.
20. Moon, H.D. *et al.* (2008) Clin. Chim. Acta **398**:99.