Angiopoietin-1 ELISA Kit (Mouse) (OKBB00588)

Lot# KF0337

Instructions for Use

For the quantitative measurement of Mouse Angiopoietin-1 in cell culture supernatants, cell lysates, serum and plasma (heparin, EDTA).

Lot to lot variations can occur. Refer to the manual provided with the kit.

This product is intended for research use only.

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1. Background

Principle

Aviva Systems Biology Angiopoietin-1 Elisa Kit (Mouse) (OKBB00588) is based on standard sandwich enzyme-linked immuno-sorbent assay technology. An antibody specific for Angiopoietin-1 has been pre-coated onto 96-wellplate (12 x 8 Well Strips). Standards (NSO; H16-F497) and test samples are added to the wells, incubated and removed. A biotinylated polyclonal goat detector antibody specific for Angiopoietin-1 is added, incubated and followed by washing. Avidin-Biotin-Peroxidase Complex is then added, incubated and unbound conjugate is washed away. An enzymatic reaction is visualized through the addition of TMB substrate which is catalyzed by HRP to produce a blue color product that changes yellow after adding acidic stop solution. The density of yellow coloration read by absorbance at 450 nm and is quantitatively proportional to the amount of sample Mouse Angiopoietin-1 captured in well.

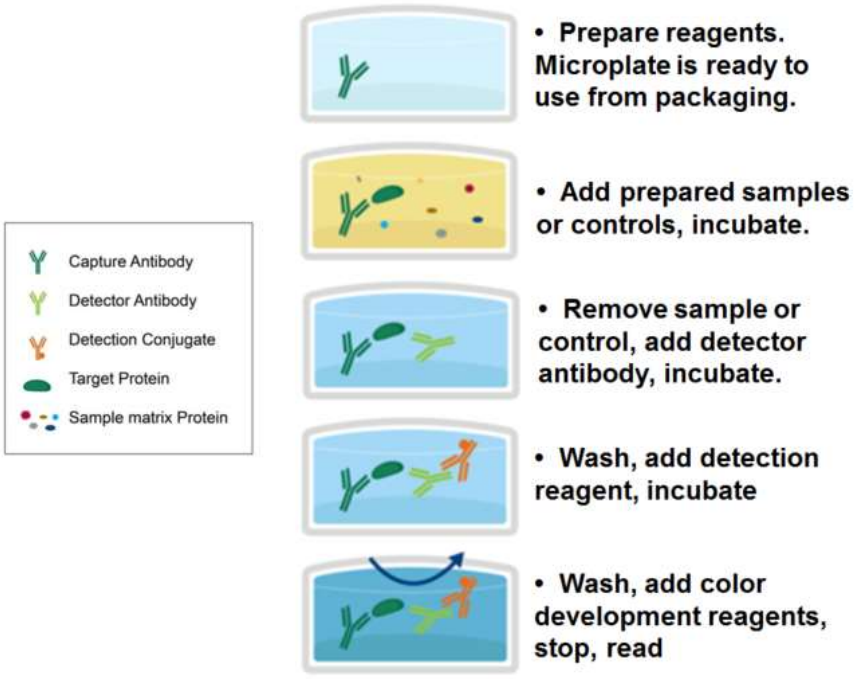
Background

Binds and activates TEK/TIE2 receptor by inducing its dimerization and tyrosine phosphorylation. Plays an important role in the regulation of angiogenesis, endothelial cell survival, proliferation, migration, adhesion and cell spreading, reorganization of the actin cytoskeleton, but also maintenance of vascular quiescence. Required for normal angiogenesis and heart development during embryogenesis. After birth, activates or inhibits angiogenesis, depending on the context. Inhibits angiogenesis and promotes vascular stability in quiescent vessels, where endothelial cells have tight contacts. In quiescent vessels, ANGPT1 oligomers recruit TEK to cell-cell contacts, forming complexes with TEK molecules from adjoining cells, and this leads to preferential activation of phosphatidylinositol 3-kinase and the AKT1 signaling cascades. In migrating endothelial cells that lack cell-cell adhesions, ANGT1 recruits TEK to contacts with the extracellular matrix, leading to the formation of focal adhesion complexes, activation of PTK2/FAK and of the downstream kinases MAPK1/ERK2 and MAPK3/ERK1, and ultimately to the stimulation of sprouting angiogenesis. Mediates blood vessel maturation/stability. Implicated in endothelial developmental processes later and distinct from that of VEGF. Appears to play a crucial role in mediating reciprocal interactions between the endothelium and surrounding matrix and mesenchyme.

General Specifications

General Specifications	
Range	0.156 – 10 ng/mL
Sensitivity	< 0.010 ng/mL (Derived by linear regression of OD_{450} of the Mean Blank + 2xSD)
Specificity	Natural and recombinant Mouse Angiopoietin-1 UniProt ID: O08538 GeneID: 11600 Alias: 1110046O21Rik, Agpt, ang1, Ang1, Ang-1, ANG-1, Angiopoietin-1
Cross-Reactivity	No detectable cross-reactivity with other relevant proteins

2. Assay Summary



3. Storage and Stability

• Upon receipt store kit at -20°C for 6 months. Avoid multiple freeze/thaw cycles.

4. Kit Components

• The following reagents are the provided contents of the kit.

Description	Quantity	Storage Conditions
96-Well Plate Pre-coated with Anti-Mouse Angiopoietin-1 Antibody	1 (12 x 8 Well Strip)	Store at -20°C for 6 months
Lyophilized Recombinant Mouse Angiopoietin-1 Standard	2 x 10 ng	
100X Biotinylated Anti-Mouse Angiopoietin-1 Antibody	1 x 100 µL	
100X Avidin-Biotin-Peroxidase Complex (ABC)	1 x 100 µL	
Sample Diluent Buffer	1 x 30 mL	
Antibody Diluent Buffer	1 x 12 mL	
ABC Diluent Buffer	1 x 12 mL	
TMB Color Developing Agent	1 x 10 mL	
TMB Stop Solution	1 x 10 mL	
10X Wash Buffer	1 x 30 mL	

5. Precautions

• Read instructions fully prior to beginning use of the assay kit.

• Any deviations or modifications from the described method or use of other reagents could result in a reduction of performance.

• Reduce exposure to potentially harmful substances by wearing personal protective lab equipment including lab coats, gloves and glasses.

• For information on hazardous substances included in the kit please refer to the Material Safety Data Sheet (MSDS).

6. Required Materials Not Supplied

• Washing Buffer (neutral PBS or TBS) – see Section 8.4 for formulation.

• Microplate reader capable of reading absorbance at 450 nm.

• Automated plate washer (optional).

• Pipettes capable of precisely dispensing 0.5 µL through 1 mL volumes of aqueous solutions.

• Pipettes or volumetric glassware capable of precisely measuring 1 mL through 100 mL of aqueous solutions.

• New, clean tubes and/or micro-centrifuge tubes for the preparation of standards or samples.

• Absorbent paper or paper toweling.

• Distilled or deionized ultrapure water.

7. Technical Application Tips

• Do not mix or substitute components from other kits.

• To ensure the validity of experimental operation, it is recommended that pilot experiments using standards and a small selection of sample dilutions to ensure optimal dilution range for quantitation.

• Samples exhibiting OD measurements higher than the highest standard should be diluted further in the appropriate sample dilution buffers.

• Inspect all reagents prior to use. Components should contain no particulates or cloudiness and should be colorless.

• Prior to using the kit, briefly spin component tubes to collect all reagent at the bottom.

• Replicate wells are recommended for standards and samples.

• Cover microplate while incubating to prevent evaporation.

• Do not allow the microplate wells dry at any point during the assay procedure.

• Do not reuse tips or tube to prevent cross contamination.

• Avoid causing bubbles or foaming when pipetting, mixing or reconstituting.

• Completely remove of all liquids when washing to prevent cross contamination.

• Prepare reagents immediately prior to use and do not store, with the exception of the top standard.

• Equilibrate all materials to ambient room temperature prior to use (standards exception).

• For optimal results in inter- intra-assay consistency, equilibrate all materials to room temperature prior to performing assay (standards exception) and perform all incubations at 37°C.

• Pipetting less than 1 µL is not recommended for optimal assay accuracy.

• Once the procedure has been started, all steps should be completed without interruption. Ensure that all reagents, materials and devices are ready at the appropriate time.

• Incubation times will affect results. All wells should be handled in the same sequential order and time intervals for optimal results.

• Samples containing precipitates or fibrin strands or which are hemolytic of lipemic might cause inaccurate results due to interfering factors.

8. Reagent Preparation

• Equilibrate all materials to room temperature prior to use and use immediately.

8.1 Angiopoietin-1 Assay Standards

8.1.1 Prepare the Angiopoietin-1 standards no greater than 2 hours prior to performing experiment. Standards should be held on ice until use in the experiment.

8.1.2 Reconstitute one of the provided 10 ng **Lyophilized Recombinant Mouse Angiopoietin-1 Standard** for each experiment. Prepare a stock 10 ng/mL **Mouse Angiopoietin-1 Standard** by reconstituting one tube of **Lyophilized Recombinant Mouse Angiopoietin-1 Standard** as follows:

8.1.2.1 Gently spin or tap the vial to collect all material at the bottom.

8.1.2.2 Add 1 mL of **Sample Diluent Buffer** to the vial.

8.1.2.3 Seal then mix gently and thoroughly.

8.1.2.4 Leave the vial to sit at ambient temperature for 10 minutes.

8.1.3 Prepare a set of seven serially diluted standards as follows:

8.1.3.1 Label tubes with numbers 2 – 8.

8.1.3.2 Add 300 µL of **Sample Diluent Buffer** to Tube #'s 2 – 8.

8.1.3.3 Use the reconstituted 10 ng/mL Mouse Angiopoietin-1 as **Standard #1**.

8.1.3.4 Prepare **Standard #2** by adding 300 µL of **10 ng/mL Standard #1** from Tube #1 to Tube #2. Mix gently and thoroughly.

8.1.3.5 Prepare **Standard #3** by adding 300 µL of **Standard #2** from Tube #2 to Tube #3. Mix gently and thoroughly.

8.1.3.6 Prepare further serial dilutions through Tube #7. Reference the table below as a guide for serial dilution scheme.

8.1.3.7 Tube #8 is a blank standard (only **Sample Diluent Buffer**), which should be included with every experiment.

Standard Number (Tube)	Sample To Dilute	Volume Standard (µL)	Volume Sample Diluent Buffer (µL)	Total Volume (µL)	Final Concentration
Tube #1	10 ng/mL of Mouse Angiopoietin-1 Standard	NA	1,000	1,000	10 ng/mL
Tube #2	10 ng/mL	300	300	600	5 ng/mL
Tube #3	5 ng/mL	300	300	600	2.5 ng/mL
Tube #4	2.5 ng/mL	300	300	600	1.25 ng/mL
Tube #5	1.25 ng/mL	300	300	600	0.625 ng/mL
Tube #6	0.625 ng/mL	300	300	600	0.313 ng/mL
Tube #7	0.313 ng/mL	300	300	600	0.156 ng/mL
Tube #8	NA	0	300	300	0.0 (Blank)

8.2 1X Biotinylated Anti-Mouse Angiopoietin-1 Antibody

8.2.1 Prepare the **1X Biotinylated Anti-Mouse Angiopoietin-1 Antibody** immediately prior to use by diluting the **100X Biotinylated Anti-Mouse Angiopoietin-1 Antibody** 1:100 with **Antibody Diluent Buffer**.

8.2.2 For each well strip to be used in the experiment (8-wells) prepare 1,000 µL by adding 10 µL of **100X Biotinylated Angiopoietin-1 Detector Antibody** to 990 µL **Antibody Diluent Buffer**.

8.2.3 Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure. Do not store at 1X concentration for future use.

8.3 1X Avidin-Biotin-Peroxidase Complex (ABC)

8.3.1 Prepare the **1X Avidin-Biotin-Peroxidase Complex (ABC)** immediately prior to use by diluting the **100X Avidin-Biotin-Peroxidase Complex (ABC)** 1:100 with **ABC Dilution Buffer**.

8.3.2 For each well strip to be used in the experiment (8-wells) prepare 1,000 µL by adding 10 µL of **100X Avidin-Biotin-Peroxidase (ABC) Complex** to 990 µL Complex **Diluent**.

8.3.3 Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure. Do not store at 1X concentration for future use.

8.4 1X Wash Buffer

8.4.1 Add 270 mL of ultra-pure water to a clean > 500 mL bottle or other vessel.

8.4.2 Add the entire 30 mL contents of the **10X Wash Buffer** bottle to the water.

8.4.3 Seal and mix gently by inversion. Avoid foaming or bubbles.

8.4.4 Store the **1X Wash Buffer at** room temperature until ready to use in the procedure. Store the prepared **1X Wash Buffer** at 4°C for no longer than 1 week. Do not freeze.

8.5 Microplate Preparation

- Micro-plates are provided ready to use and do not require rinsing or blocking.
- Unused well strips should be returned to the original packaging, sealed and stored at 4°C.
- Equilibrate microplates to ambient temperatures prior to opening to reduce potential condensation.

9. Sample Preparation

9.1 Sample Preparation and Storage

• Store samples to be assayed at 2-8°C for 24 hours prior being assayed.

• For long term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

• Clear samples by centrifugation as follows:

• **Cell culture supernatant** - Remove particulates by centrifugation, analyze immediately or aliquot and store at -20°C. For cell lysate, add lysis solution before centrifugation.

• **Serum** - Allow the serum to clot in a serum separator tube (about 4 hours) at room temperature. Centrifuge at approximately 2,000 x g for 20 min. Analyze the serum immediately or aliquot and store frozen at -20°C.

• **Plasma** - Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 20 min at 2,000 x g within 30 min of collection at 2-8°C. Analyze immediately or aliquot and store frozen at -20°C.

• **Cell Lysates** – Lyse the cells, make sure there are no visible cell sediments. Centrifuge cell lysates at approximately 10,000 x g for 5 min. Collect supernatant.

9.2 Sample Dilution

Target protein concentration must be estimated and appropriate sample dilution selected such that the final target protein concentration falls near the middle of the assay linear dynamic range.

• Prepare 150 µL sample for each replicate to be assayed.

• Dilute samples with **Sample Diluent Buffer**.

• Mix diluted samples gently and thoroughly.

• Pipetting less than 2 µL is not recommended for optimal assay accuracy.

• Refer to the following table for recommended sample dilution guidelines based on the dynamic range of this kit:

Estimated Sample Target Concentration		Dilution Level	Sample Volume For Two Replicates	Sample Diluent Buffer For Two Replicates
High Concentration	100-1,000 ng/mL	1:100	3 µL	297 µL
Medium Concentration	10-100 ng/mL	1:10	30 µL	270 µL
Low Concentration	156-10,000 pg/mL	1:2	150 µL	150 µL
Very Low Concentration	≤156 pg/mL	1:2 or No Dilution	-	-

10. Assay Procedure

• The ABC Working Solution, TMB Color Developing Agent and TMB Stop Solution must be kept warm at 37°C for 30 minutes prior to use. Equilibrate all other reagents and materials to ambient room temperature prior to use in the procedure.

• To control for small potential variations in micro well-plate and day to day ambient temperature fluctuations, equilibrate all reagents prior to use and perform all incubation steps at to 37°C for optimal consistency and reproducibility.

10.1 Add 100 µL of serially titrated standards, diluted samples or blank into wells of the pre-coated well plate. At least two replicates of each standard, sample or blank is recommended.

10.2 Cover the plate with the plate sealer and incubate at 37°C for 90 minutes.

10.3 Remove the plate sealer and discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.

10.4 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.5 Add 100 µL of prepared **1X Biotinylated Anti-Human Angiopoietin-1 Antibody to each well.**

10.6 Cover with the plate sealer and incubate at 37°C for 60 minutes.

10.7 Wash plate 3 times with **1X Wash Buffer as** follows:

10.7.1 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.

10.7.2 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.7.3 Add 300 µL of **1X Wash Buffer** to each assay well.

10.7.4 Incubate for 1 minute.

10.7.5 Repeat steps 10.7.1 through 10.7.4 **two** more times.

10.8 Add 100 µL of prepared **1X Avidin-Biotin-Peroxidase Complex (ABC) into** each well, cover with plate sealer and incubate at 37°C for 30 minutes.

10.9 Wash plate 5 times with **1X Wash Buffer as** follows:

10.9.1 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.

10.9.2 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.9.3 Add 300 µL of 1X Wash Buffer to each assay well.

10.9.4 Incubate for 1 minute.

10.9.5 Repeat steps 10.9.1 through 10.9.4 **four** more times.

10.10 Add 90 µL of **TMB Color Developing Agent** to each well, cover with plate sealer and incubate at 37°C in the dark for 15-25 minutes.

(NOTE: optimal incubation time must be determined by the user. Optimal development can be visualized by blue shading in the top four standard wells, while the remaining standards are still clear.)

10.11 Add 100 µL of **TMB Stop Solution** to each well. Well color should change to yellow immediately.

10.12 Read the O.D. absorbance at 450 nm with a standard microplate reader within 30 minutes of stopping the reaction in step 10.11.

11. Calculation of Results

For analysis of the assay results, calculate the **Relative OD_{450}** for each test or standard well as follows:

$$(\text{Relative } OD_{450}) = (\text{Well } OD_{450}) - (\text{Mean Blank Well } OD_{450})$$

The standard curve is generated by plotting the mean replicate **Relative OD_{450}** of each standard serial dilution point vs. the respective standard concentration. The Mouse Angiopoietin-1 concentration contained in the samples can be interpolated by using linear regression of each mean sample **Relative OD_{450}** against the standard curve. This is best achieved using curve fitting software.

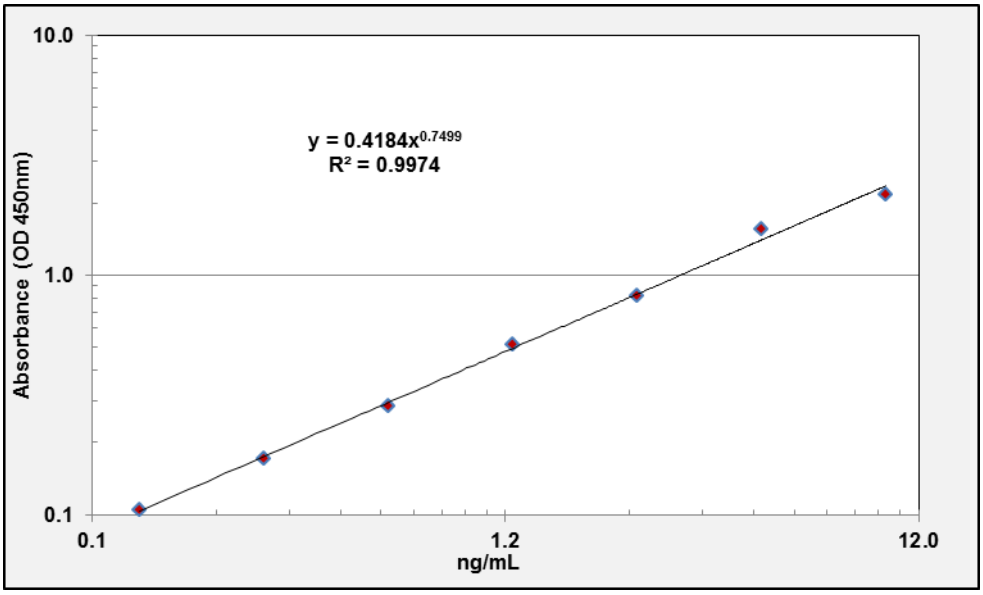
Note: if the samples measured were diluted, multiply the derived mean sample concentration by the dilution factor for a final sample concentration.

12. Typical Expected Data

12.1 Typical absorbance values. Expected absorbance for standards when TMB incubation is performed for 20 minutes at 37°C and measured at OD_{450}.

Standard Number	8	7	6	5	4	3	2	1
Concentration (ng/mL)	0	0.156	0.313	0.625	1.25	2.5	5.0	10
OD_{450}	0.053	0.105	0.171	0.285	0.513	0.819	1.549	2.174

12.2 Typical standard curve. This standard curve is for demonstration purposes only. An assay specific standard curve should be performed with each assay.



12.3 Reproducibility

	Intra-Assay			Inter-Assay		
Sample ID	1	2	3	1	2	3
n =	16	16	16	24	24	24
Mean (pg/mL)	351	1479	5183	351	1592	4701
Standard Deviation	27.02	97.61	207.32	30.53	117.8	230.34
Consistency (%CV)	7.7	6.6	4.0	8.7	7.4	4.9

13. Technical Resources

References

13.1.1 Fukuhara, S., Sako, K., Minami, T., Noda, K., Kim, H. Z., Kodama, T., Shibuya, M., Takakura, N., Koh, G. Y., Mochizuki, N. Differential function of Tie2 at cell-cell contacts and cell-substratum contacts regulated by Angiopoietin-1. Nature Cell Biol. 10: 513-526, 2008.

13.1.2 Ward, E. G., Grosios, K., Markham, A. F., Jones, P. F. Genomic structures of the human angiopoietins show polymorphism in angiopoietin-2. Cytogenet. Cell Genet. 94: 147-154, 2001.

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