



Rat Angiopoietin-2 ELISA Kit

Cat #: orb385410 (manual)

Sandwich Enzyme-Linked Immunosorbent Assay for Quantitative Detection of Rat Angiopoietin-2 Concentrations in Cell Culture Supernates, Serum, Plasma.

For research use only. Not for diagnostic or therapeutic procedures.



INTRODUCTION

Angiopoietin-2 is a protein that in humans is encoded by the ANGPT2 gene. Angiopoietins are members of the vascular endothelial growth factor family and the only known growth factors largely specific for vascular endothelium. ANGPT2 is a naturally occurring antagonist of angiopoietin-1 that competes for binding to the TIE2 receptor and blocks ANGPT1-induced TIE2 autophosphorylation during vasculogenesis. Its expression occurs at sites of vascular remodelling: dorsal aorta and major aortic branches, ovary, placenta and uterus.

ASSAY PRINCIPLES

The Biorbyt Rat Angiopoietin-2 ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of Rat Angiopoietin-2 in Cell Culture Supernates, Serum, Plasma. This assay employs an antibody specific for Rat Angiopoietin-2 coated on a 96-well plate. Standards and samples are pipetted into the wells and Angiopoietin-2 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-rat Angiopoietin-2 antibody is added. After washing away unbound biotinylated antibodies, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of Angiopoietin-2 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.





KIT COMPONENTS

Component	Volume
96-well Plate Coated With Anti-Rat Angiopoietin-2 Antibody	8 wells x 12 Strips
Rat Angiopoietin-2 Standard	10 ng x 2
Biotin-Labeled Detection Antibody (100X)	120 μl
Streptavidin-HRP (100X)	120 μl
Standard/Sample Diluent	30 ml
Detection Antibody Diluent	12 ml
Streptavidin-HRP Diluent	12 ml
Wash Buffer (20X)	30 ml
TMB Substrate Solution	12 ml
Stop Solution	12 ml
Plate Adhesive Strips	3 Strips
Technical Manual	1 Manual

STORAGE AND STABILITY

All kit components are stable at 2 to 8 °C. Standard (recombinant protein) should be stored at -20 °C or -80 °C (recommended at -80 °C) after reconstitution. Opened Microplate Wells or reagents may be stored for up to 1 month at 2 to 8 °C. Return unused wells to the pouch containing the desiccant pack, and reseal along the entire edge.

Note: the kit can be used within one year if the whole kit is stored at -20 °C. Avoid repeated freeze-thaw cycles.



MATERIALS REQUIRED BUT NOT PROVIDED

- 1. Microplate reader capable of measuring absorbance at 450 nm.
- 2. Adjustable pipettes and pipette tips to deliver 2 µl to 1 ml volumes.
- 3. Adjustable 1-25 ml pipettes for reagent preparation.
- 4. 100 ml and 1-liter graduated cylinders.
- 5. Absorbent paper.
- 6. Distilled or deionized water.
- 7. Computer and software for ELISA data analysis.
- 8. Tubes to prepare standard or sample dilutions.

HEALTH AND SAFETY PRECAUTIONS

- 1. Reagents provided in this kit may be harmful if ingested, inhaled, or absorbed through the skin. Please carefully review the MSDS for each reagent before conducting the experiment.
- 2. Stop Solution contains 2 N Sulfuric Acid (H₂SO₄) and is an extremely corrosive agent. Please wear proper eye, hand, and face protection when handling this material. When the experiment is finished, be sure to rinse the plate with copious amounts of running water to dilute the Stop Solution before disposing of the plate.
- 3. Standard protein and Detection Antibody containing Sodium Azide as a preservative.



REAGENT PREPARATION

1. Sample Preparation

Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

Cell culture supernates: Remove particulates by centrifugation, assay immediately or aliquot, and store samples at -20°C.

Serum: Allow the serum to clot in a serum separator tube (about 4 hours) at room temperature. Centrifuge at approximately 1000 X g for 15 minutes. Analyze the serum immediately or aliquot and store samples at -20°C.

Plasma: Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1500 X g within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C.

Cell Lysates: Collect cells and rinse cells with PBS. Homogenize and lysate cells thoroughly in lysate solution. Centrifuge cell lysates at approximately 10000 X g for 5 minutes to remove debris. Aliquots of the cell lysates were removed and assayed.

Bone Tissue: Extract demineralized bone samples in 4 M Guanidine-HCl and protease inhibitors. Dissolve the final sample in 2 M Guanidine-HCl.

Tissue Homogenates: The preparation of tissue homogenates will vary depending upon tissue type. Rinse tissue with 1X PBS to remove excess blood, homogenized in 20 mL of 1X PBS, and stored overnight at \leq -20 °C. After two freeze-thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 5 minutes at 5000 x g. The supernate was removed immediately and assayed. Alternatively, aliquot and store samples at \leq -20 °C.

Note: Some lysis buffers, such as RIPA can not be used. Some components will affect the binding. **Urine:** Urinary samples should be cleared by centrifugation and then can be used directly without dilution. Storage at -20°C.

2. Rat Angiopoietin-2 Standard Preparation

Reconstitute the lyophilized Rat Angiopoietin-2 Standard by adding 1 ml of Standard/Sample Diluent to make the 10000 pg/ml standard stock solution. Allow the solution to sit at room temperature for 5 minutes, then gently vortex to mix completely. Use within one hour of reconstituting. Two tubes of the standard (10 ng per tube) are included in each kit. Use one tube for each experiment.

Perform 2-fold serial dilutions of the top standards to make the standard curve within the range of this assay (156 pg/ml - 10000 pg/ml) as below. Standard/Sample Dilution Buffer serves as the zero standard (0 pg/ml).





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Standard	Add	Into
10000 pg/ml		
5000 pg/ml	500 μl of the Standard (10000 pg/ml)	500 µl of the Standard/Sample Diluent
2500 pg/ml	500 μl of the Standard (5000 pg/ml)	500 µl of the Standard/Sample Diluent
1250 pg/ml	500 μl of the Standard (2500 pg/ml)	500 μl of the Standard/Sample Diluent
625 pg/ml	500 μl of the Standard (1250 pg/ml)	500 μl of the Standard/Sample Diluent
312 pg/ml	500 μl of the Standard (625 pg/ml)	500 μl of the Standard/Sample Diluent
156 pg/ml	500 μl of the Standard (312 pg/ml)	500 μl of the Standard/Sample Diluent
0 pg/ml	1 ml of the Standard/Sample Diluent	

Note: The standard solutions are best used within 2 hours. The 10000 pg/ml standard solution should be stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.

3. Biotin-Labeled Detection Antibody Working Solution Preparation

The Biotin-Labeled Detection Antibody should be diluted in 1: 100 with the Detection Antibody Diluent and mixed thoroughly. The solution should be prepared no more than 2 hours prior to the experiment.

4. Streptavidin-HRP Working Solution Preparation

The Streptavidin-HRP should be diluted in 1: 100 with the Streptavidin-HRP Diluent and mixed thoroughly. The solution should be prepared no more than 1 hour prior to the experiment.

5. Wash Buffer Working Solution Preparation

Pour entire contents (30 ml) of the Wash Buffer Concentrate into a clean 1, 000 ml graduated cylinder. Bring the final volume to 600 ml with glass-distilled or deionized water (1: 20).



ASSAY PROCEDURE

The Streptavidin-HRP Working Solution and TMB Substrate Solution must be kept warm at 37°C for 30 minutes before use. When diluting samples and reagents, they must be mixed completely and evenly. A standard detection curve should be prepared for each experiment. The user will decide on sample dilution fold by crude estimation of protein amount in samples.

- 1. Add 100 µl of each standard and sample into appropriate wells.
- 2. Cover well and incubate for 90 minutes at room temperature or overnight at 4°C with gentle shaking.
- 3. Remove the cover, discard the solution, and wash the plate 3 times with Wash Buffer Working Solution, and each time let Wash Buffer Working Solution stay in the wells for 1 2 minutes. Blot the plate onto paper towels or other absorbent material. Do NOT let the wells completely dry at any time.
- 4. Add 100 μl of Biotin-Labeled Detection Antibody Working Solution into each well and incubate the plate at 37°C for 60 minutes.
- 5. Wash the plate 3 times with Wash Buffer Working Solution, and each time let Wash Buffer Working Solution stay in the wells for 1 2 minutes. Discard the Wash Buffer Working Solution and blot the plate onto paper towels or other absorbent material.
- 6. Add 100 μl of Streptavidin-HRP Working Solution into each well and incubate the plate at 37°C for 45 minutes.
- 7. Wash the plate 5 times with Wash Buffer Working Solution, and each time let the wash buffer stay in the wells for 1 2 minutes. Discard the wash buffer and blot the plate onto paper towels or other absorbent material.
- 8. Add 100 μ l of TMB Substrate Solution into each well and incubate the plate at 37°C in the dark for 10-20 minutes.
- 9. Add 100 µl of Stop Solution into each well. The color changes into yellow immediately.
- 10. Read the O.D. absorbance at 450nm in a microplate reader within 30 minutes after adding the Stop Solution.

For calculation, (the relative O.D.450) = (the O.D.450 of each well) - (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The concentration of the samples can be interpolated from the standard curve.

Note: If the samples measured were diluted, multiply the dilution factor by the concentrations from interpolation to obtain the concentration before dilution.



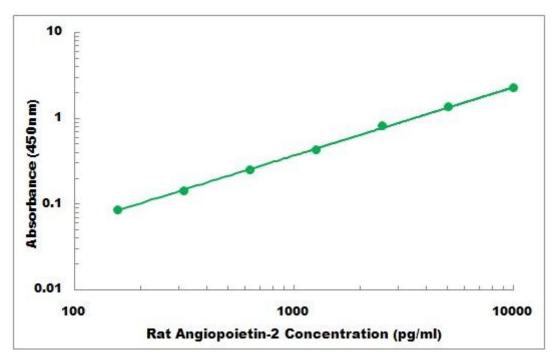
ASSAY PROCEDURE SUMMARY

- Prepare all reagents, samples and standards
- Add 100 µl Standard or Sample
- Wash plate 3 times with Wash Buffer Working Solution
- Add 100 µl Biotin-Labeled Detection Antibody Working Solution
- Wash plate 3 times with Wash Buffer Working Solution
- Add 100 μl Streptavidin-HRP Working Solution
- Wash plate 5 times with Wash Buffer Working Solution
- Add 100 μl TMB Substrate Solution
- Add 100 μl Stop Solution
- Read the plate at 450nm



TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



SENSITIVITY

The minimum detectable dose of Rat Angiopoietin-2 is typically less than 15 pg/ml.

SPECIFICITY

The Rat Angiopoietin-2 ELISA Kit allows for the detection and quantification of endogenous levels of natural and/or recombinant Rat Angiopoietin-2 proteins within the range of 156 pg/ml - 10000 pg/ml.





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CROSS REACTIVITY

No detectable cross-reactivity with other relevant proteins.

TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
High signal and background in	Insufficient washing	Increase number of washes
all wells		• Increase the time of soaking
		between in-wash
	Too much Streptavidin-HRP	Check dilution, titration
	• Incubation time too long	Reduce incubation time
	Development time too long	Decrease the incubation time
		before the stop solution is added
No signal	• Reagent added in incorrect order	Review protocol
	or incorrectly prepared	
	• Standard has gone bad (If there is	Check the condition of stored
	a signal in the sample wells)	standard
	Assay was conducted from an	Reagents are allowed to come
	incorrect starting point	to 20 - 30 °C before performing
		the assay
Too much signal-whole plate	• Insufficient washing-unbound	• Increase the number of washes
turned uniformly blue	Streptavidin-HRP remaining	carefully
	Too much Streptavidin-HRP	Check dilution
	• Plate sealer or reservoir reused,	Use fresh plate sealer and
	resulting in the presence of	reagent reservoir for each step
	residual Streptavidin-HRP	
Standard curve achieved but	Plate not developed long enough	Increase substrate solution
poor discrimination between		incubation time
point	• Improper calculation of standard	Check dilution, make a new
	curve dilution	standard curve
No signal when a signal is	Sample matrix is masking	More diluted sample
expected, but the standard curve	detection	Recommended
looks fine		
Samples are reading too high,	Samples contain protein levels	• Dilute samples and run Again
but the standard curve is fine	above the assay range	
Edge effect	• Uneven temperature around the	Avoid incubating plates in
	work surface	areas where environmental
		conditions vary
		• Use plate sealer