

# **ab171335 – Mouse Angiopoietin-2 ELISA Kit**

With Wash Buffer (25x)

## Instructions for Use

For quantitative detection of mouse Angiopoietin-2 in cell culture supernatants, cell lysates, serum and plasma (heparin, EDTA).

This product is for research use only and is not intended for diagnostic use.

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## 1. BACKGROUND

Abcam's mouse Angiopoietin-2 *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of mouse Angiopoietin-2 in cell culture supernatants, cell lysates, serum and plasma (heparin, EDTA).

An Angiopoietin-2 specific rat monoclonal antibody has been precoated onto 96-well plates. Standards and test samples are added to the wells and incubated. A biotinylated detection polyclonal antibody from goat, specific for Angiopoietin-2 is then added followed by washing with 1X Wash Buffer. Avidin-Biotin-Peroxidase Complex is added and unbound conjugates are washed away with 1X Wash Buffer. TMB is then used to visualize the HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue color product that changes into yellow after adding acidic stop solution. The density of yellow coloration is directly proportional to the mouse Angiopoietin-2 amount of sample captured in plate.

Angiopoietin-2 is a protein that in Humans is encoded by the ANGPTL2 gene. Angiopoietins are members of the vascular endothelial growth factor family and the only known growth factors largely specific for vascular endothelium. Angiopoietin-1, angiopoietin-2, and angiopoietin-4 participate in the formation of blood vessels. Human and mouse ANGPT2 are 85% identical in amino acid sequence and approximately 60% identical to their ANGPT1 homologs. Transgenic overexpression of ANGPT2 in mice disrupted blood vessel formation in the mouse embryo. By FISH and radiation hybrid analysis, the Human ANGPT2 gene was mapped to 8p23, and in a region of syntenic homology on mouse chromosome 8, where the *Angpt2* gene was mapped. 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.

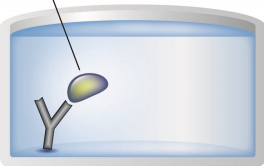
## 2. ASSAY SUMMARY

**Primary Capture Antibody**



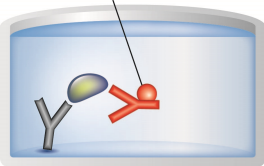
Prepare all reagents, samples and standards as instructed.

**Sample**



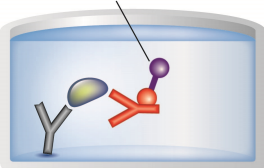
Add standard or sample to each well used. Incubate at room temperature.

**Biotinylated Antibody**



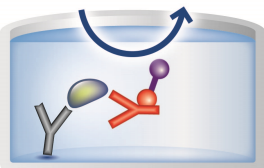
Add prepared biotin antibody to each well. Incubate at room temperature.

**Avidin-Biotin-Peroxidase Complex**



Add prepared Avidin-Biotin-Peroxidase Complex (ABC). Incubate at room temperature.

**Substrate      Colored Product**



Add TMB Color Developing Agent to each well. Incubate at room temperature. Add Stop Solution to each well. Read

## 3. PRECAUTIONS

**Please read these instructions carefully prior to beginning the assay.**

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

## 4. STORAGE AND STABILITY

**Store kit at -20°C immediately upon receipt. Avoid multiple freeze-thaw cycles.**

Refer to list of materials supplied for storage conditions of individual components.

## 5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
Anti-mouse Angiopoietin-2 Microplate (12 x 8 wells)	96 Wells	-20°C
Lyophilized recombinant mouse Angiopoietin-2 standard	2 x 10 ng	-20°C
Biotinylated anti-mouse Angiopoietin-2 antibody	100 µL	-20°C
Avidin-Biotin-Peroxidase Complex (ABC)	100 µL	-20°C
Sample Diluent Buffer	30 mL	-20°C
Antibody Diluent Buffer	12 mL	-20°C
ABC Diluent Buffer	12 mL	-20°C
TMB Color Developing Agent	10 mL	-20°C
TMB Stop Solution	10 mL	-20°C
Plate seals	4 units	-20°C
Wash Buffer (25X)	20 mL	-20°C

### **6. MATERIALS REQUIRED, NOT SUPPLIED**

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Standard microplate reader
- Automated plate washer (optional)
- Adjustable pipettes and pipette tips. Multichannel pipettes are recommended when large sample sets are being analyzed
- Eppendorf tubes

### **7. LIMITATIONS**

- Assay kit intended for research use only. Not for use in diagnostic procedures
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted

### 8. TECHNICAL HINTS

- To determine the appropriate sample dilution to use in this ELISA a pilot experiment using standards and a small number of samples is recommended
- The TMB Color Developing agent is colorless and transparent before use
- Before using the kit, briefly centrifuge the tubes in case any of the contents are trapped in the lid
- It is recommended to assay all standards, controls and samples in duplicate
- Do not let the 96-well plate dry out as this will inactivate active components on plate
- To avoid cross contamination do not reuse tips and tubes
- In order to avoid marginal effects of plate incubation due to temperature difference (reaction may be stronger in the marginal wells), it is suggested that the diluted ABC and TMB solution be pre-warmed in 37°C for 30 minutes before using
- **This kit is sold based on number of tests. A ‘test’ simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions**

## 9. REAGENT PREPARATION

Equilibrate all reagents and samples to room temperature (18 - 25°C) prior to use.

### 9.1 1X Biotinylated anti-mouse Angiopoietin-2

Biotinylated anti-mouse Angiopoietin-2 antibody must be diluted 1:100 with the Antibody Diluent Buffer and mixed thoroughly (i.e. add 1  $\mu$ L Biotinylated anti-mouse Angiopoietin antibody to 99  $\mu$ L Antibody Diluent Buffer.) The total volume required should be; 100  $\mu$ L/well multiplied by the total number of wells (allow 100  $\mu$ L - 200  $\mu$ L extra for pipetting error).

### 9.2 1X Avidin-Biotin-Peroxidase Complex

Avidin-Biotin-Peroxidase Complex (ABC) must be diluted 1:100 with ABC Diluent Buffer and mixed thoroughly (i.e. add 1  $\mu$ L ABC to 99  $\mu$ L ABC Diluent Buffer.) The total volume required should be; 100  $\mu$ L/well multiplied by the total number of wells (allow 100  $\mu$ L - 200  $\mu$ L extra for pipetting error).

### 9.3 1X Wash Buffer

Prepare 500 mL of working 1X Wash Buffer by diluting 20 ml of the supplied Wash Buffer (25X) with 480 ml of deionized or distilled water. If crystals have formed in the concentrate, warm to room temperature and mix it gently until crystals have completely dissolved.

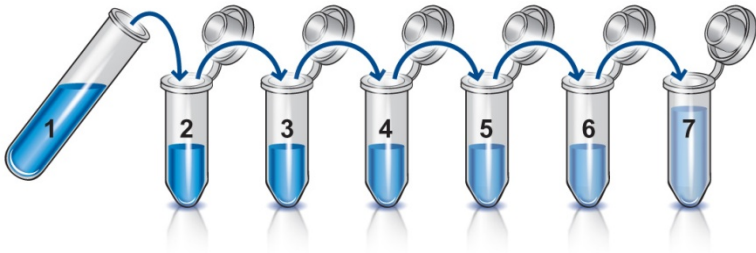
## 10. STANDARD PREPARATIONS

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use. Reconstitution of the mouse Angiopoietin-2 standard should be prepared no more than 2 hours prior to the experiment. Two tubes of Angiopoietin-2 standard (10 ng per tube) are included in each kit. Use one tube for each experiment.

- 10.1 Prepare a 10 ng/mL **Standard #1** by reconstituting the Angiopoietin-2 standard with addition of 1 mL Sample Diluent Buffer. Hold at room temperature for 10 minutes. This 10 ng/mL **Standard #1** 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.
- 10.2 Label seven tubes with #2 - 8.
- 10.3 Add 300 µL Sample Diluent Buffer into tubes #2 - 8.
- 10.4 Prepare **Standard #2** by transferring 300 µL from Standard #1 to tube #2. Mix thoroughly and gently.
- 10.5 Prepare **Standard #3** by transferring 300 µL from Standard #2 to tube #3. Mix thoroughly and gently.
- 10.6 Prepare **Standard #4** by transferring 300 µL from Standard #3 to tube #4. Mix thoroughly and gently.
- 10.7 Using the table below as a guide, repeat for tubes #5 through #7.
- 10.8 **Standard #8** contains no protein and is the Blank control.

# ASSAY PREPARATION

Standard #	Sample to Dilute	Volume to Dilute ( $\mu\text{L}$ )	Volume of Diluent ( $\mu\text{L}$ )	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	<b>Step 10.1</b>				<b>10,000</b>
2	Standard #1	300	300	10,000	5,000
3	Standard #2	300	300	5,000	2,500
4	Standard #3	300	300	2,500	1,250
5	Standard #4	300	300	1,250	625
6	Standard #5	300	300	625	312
7	Standard #6	300	300	312	156
8	None	-	300	-	-



## **11. SAMPLE COLLECTION AND STORAGE**

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.

### **11.1 Cell Culture Supernatants**

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

### **11.2 Cell Lysates**

After sufficient splitting, there should be no obvious cell sediment. Centrifuge cell lysates at approximately 10,000 x *g* for 5 minutes. Collect the cell lysate supernatants to go ahead.

### **11.3 Serum**

Allow the serum to clot in a serum separator tube (about 4 hours) at room temperature. Centrifuge at approximately 1,000 x *g* for 15 minutes. Analyze the serum immediately or aliquot and store samples at -20°C.

### **11.4 Plasma**

Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1,000 x *g* within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C.

## 12. SAMPLE PREPARATION

### **General Sample information:**

The user needs to estimate the concentration of the target protein in the sample and select the correct dilution factor so that the diluted target protein concentration falls near the middle of the linear regime in the standard curve.

Dilute the samples using the provided Sample Diluent Buffer. The following is a guideline for sample dilution. Several trials may be necessary to determine the optimal dilution factor. The sample must be thoroughly mixed with the Sample Diluent Buffer before assaying.

- High target protein concentration (100 - 1000 ng/mL). The working dilution is 1:100. i.e. Add 1  $\mu$ L sample into 99  $\mu$ L Sample Diluent Buffer
- Medium target protein concentration (10 - 100 ng/mL). The working dilution is 1:10. i.e. Add 10  $\mu$ L sample into 90  $\mu$ L Sample Diluent Buffer
- Low target protein concentration (156 - 10,000 pg/mL). The working dilution is 1:2. i.e. Add 50  $\mu$ L sample to 50  $\mu$ L Sample Diluent Buffer
- Very Low target protein concentration ( $\leq$  156 pg/mL). No dilution necessary, or the working dilution is 1:2.

## **13. PLATE PREPARATION**

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents
- Unused well strips should be returned to the plate packet and stored at 4°C
- For each assay performed, a minimum of 2 wells must be used as blanks, omitting primary antibody from well additions
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates)
- Well effects have not been observed with this assay. Contents of each well can be recorded on the template sheet included in the Resources section

## **14. ASSAY PROCEDURE**

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

14.1 Prepare all reagents, working standards, and samples as directed in the previous sections

14.2 Add 100  $\mu$ L of prepared standards and diluted samples to appropriate wells.

14.3 Seal the plate with a new plate seal and incubate at 37°C for 90 minutes.

14.4 Remove the seal, discard contents of each well, and blot the plate onto paper towels or other absorbent material. Do NOT let the wells completely dry at any time.

14.5 Add 100  $\mu$ L of 1X Biotinylated anti-mouse Angiopoietin-2 antibody into each well and incubate the plate at 37°C for 60 minutes.

14.6 Wash the plate three times with 300  $\mu$ L of 1X Wash Buffer, and each time let the washing buffer stay in the wells for one minute. Discard the washing buffer and blot the plate onto paper towels or other absorbent material.

*Note:* For automated washing, aspirate all wells and wash THREE times with 1X Wash Buffer, overfilling wells with each wash. Blot the plate onto paper towels or other absorbent material.

14.7 Add 100  $\mu$ L of 1X Avidin-Biotin-Peroxidase Complex working solution into each well and incubate the plate at 37°C for 30 minutes.

14.8 Wash plate five times with 1X Wash Buffer, and each time let washing buffer stay in the wells for 1 - 2 minutes. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (See Step 14.6 for plate washing method).

- 14.9 Add 90  $\mu\text{L}$  of prepared TMB color developing agent into each well and incubate plate at 37°C in dark for 15 – 25 minutes.

*Note:* The optimal incubation time should be determined by end user. The shades of blue should be seen in the wells with the four most concentrated mouse Angiopoietin standard solutions; the other wells show no obvious color.

- 14.10 Add 100  $\mu\text{L}$  of prepared TMB Stop Solution into each well. The color changes into yellow immediately.
- 14.11 Read the O.D. absorbance at 450 nm in a microplate reader within 30 minutes after adding the stop solution.

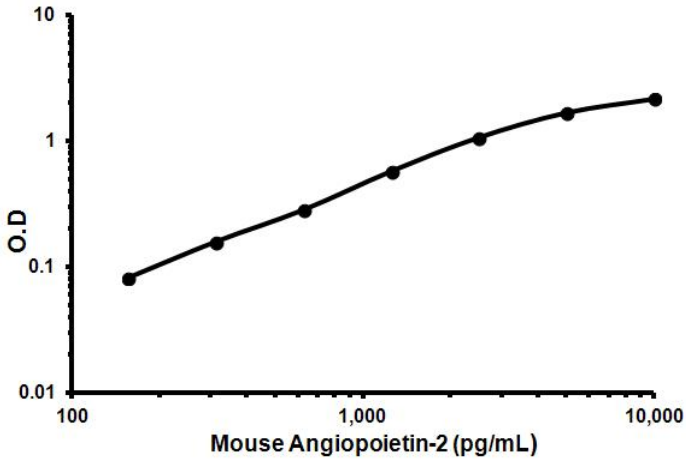
## 15. CALCULATIONS

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 mouse Angiotensin-2 concentration of the samples can be interpolated from the standard curve.

Note: If the samples measured were diluted, make sure to account for this in your calculations.

## 16. TYPICAL DATA

**TYPICAL STANDARD CURVE** – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.



Conc. (pg/mL)	O.D. 450nm
0.0	0.009
156	0.082
312	0.159
625	0.285
1,250	0.578
2,500	1.073
5,000	1.689
10,000	2.174

### **17. TYPICAL SAMPLE VALUES**

**RANGE** – 156 - 10,000 pg/mL

**SENSITIVITY** – < 10 pg/mL

### **18. ASSAY SPECIFICITY**

This kit detects both endogenous and recombinant mouse Angiotensin-2.

No detectable cross-reactivity with other relevant proteins.

## 19. TROUBLESHOOTING

<b>Problem</b>	<b>Cause</b>	<b>Solution</b>
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standards dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Samples give higher value than the highest standard	Starting sample concentration is too high.	Dilute the specimens and repeat the assay
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the kit	Store the all components as directed.

20. NOTES









## Technical Support

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For all technical or commercial enquiries please go to:

[www.abcam.com/contactus](http://www.abcam.com/contactus)

[www.abcam.cn/contactus](http://www.abcam.cn/contactus) (China)

[www.abcam.co.jp/contactus](http://www.abcam.co.jp/contactus) (Japan)

# **ab171335 – Mouse Angiopoietin-2 ELISA Kit**

Without Wash Buffer (25x)

## Instructions for Use

For quantitative detection of mouse Angiopoietin-2 in cell culture supernatants, cell lysates, serum and plasma (heparin, EDTA).

This product is for research use only and is not intended for diagnostic use.

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## 1. BACKGROUND

Abcam's mouse Angiopoietin-2 *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of mouse Angiopoietin-2 in cell culture supernatants, cell lysates, serum and plasma (heparin, EDTA).

An Angiopoietin-2 specific rat monoclonal antibody has been precoated onto 96-well plates. Standards and test samples are added to the wells and incubated. A biotinylated detection polyclonal antibody from goat, specific for Angiopoietin-2 is then added followed by washing with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex is added and unbound conjugates are washed away with PBS or TBS buffer. TMB is then used to visualize the HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue color product that changes into yellow after adding acidic stop solution. The density of yellow coloration is directly proportional to the mouse Angiopoietin-2 amount of sample captured in plate.

Angiopoietin-2 is a protein that in Humans is encoded by the ANGPTL2 gene. Angiopoietins are members of the vascular endothelial growth factor family and the only known growth factors largely specific for vascular endothelium. Angiopoietin-1, angiopoietin-2, and angiopoietin-4 participate in the formation of blood vessels. Human and mouse ANGPT2 are 85% identical in amino acid sequence and approximately 60% identical to their ANGPT1 homologs. Transgenic overexpression of ANGPT2 in mice disrupted blood vessel formation in the mouse embryo. By FISH and radiation hybrid analysis, the Human ANGPT2 gene was mapped to 8p23, and in a region of syntenic homology on mouse chromosome 8, where the *Angpt2* gene was mapped. 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.

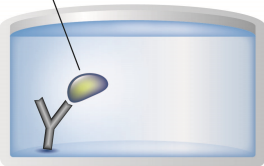
## 2. ASSAY SUMMARY

**Primary Capture Antibody**



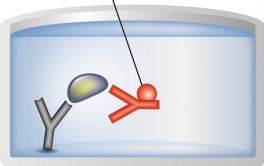
Prepare all reagents, samples and standards as instructed.

**Sample**



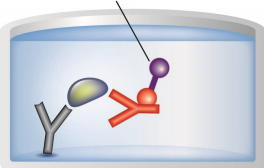
Add standard or sample to each well used. Incubate at room temperature.

**Biotinylated Antibody**



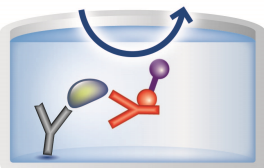
Add prepared biotin antibody to each well. Incubate at room temperature.

**Avidin-Biotin-Peroxidase Complex**



Add prepared Avidin-Biotin-Peroxidase Complex (ABC). Incubate at room temperature.

**Substrate      Colored Product**



Add TMB to each well. Incubate at room temperature. Add Stop Solution to each well. Read

## 3. PRECAUTIONS

**Please read these instructions carefully prior to beginning the assay.**

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

## 4. STORAGE AND STABILITY

**Store kit at -20°C immediately upon receipt. Avoid multiple freeze-thaw cycles.**

Refer to list of materials supplied for storage conditions of individual components.

## 5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
Anti-mouse Angiopoietin-2 Microplate (12 x 8 wells)	96 Wells	-20°C
Lyophilized recombinant mouse Angiopoietin-2 standard	2 x 10 ng	-20°C
Biotinylated anti-mouse Angiopoietin-2 antibody	100 µL	-20°C
Avidin-Biotin-Peroxidase Complex (ABC)	100 µL	-20°C
Sample Diluent Buffer	30 mL	-20°C
Antibody Diluent Buffer	12 mL	-20°C
ABC Diluent Buffer	12 mL	-20°C
TMB Color Developing Agent	10 mL	-20°C
TMB Stop Solution	10 mL	-20°C
Plate seals	4 units	-20°C

### **6. MATERIALS REQUIRED, NOT SUPPLIED**

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Standard microplate reader
- Automated plate washer (optional)
- Adjustable pipettes and pipette tips. Multichannel pipettes are recommended when large sample sets are being analyzed
- Eppendorf tubes
- Washing buffer, either neutral PBS or TBS (see Section 9 for recipes)

### **7. LIMITATIONS**

- Assay kit intended for research use only. Not for use in diagnostic procedures
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted

### 8. TECHNICAL HINTS

- To determine the appropriate sample dilution to use in this ELISA a pilot experiment using standards and a small number of samples is recommended
- The TMB Color Developing agent is colorless and transparent before use
- Before using the kit, briefly centrifuge the tubes in case any of the contents are trapped in the lid
- It is recommended to assay all standards, controls and samples in duplicate
- Do not let the 96-well plate dry out as this will inactivate active components on plate
- To avoid cross contamination do not reuse tips and tubes
- In order to avoid marginal effects of plate incubation due to temperature difference (reaction may be stronger in the marginal wells), it is suggested that the diluted ABC and TMB solution be pre-warmed in 37°C for 30 minutes before using
- **This kit is sold based on number of tests. A ‘test’ simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions**

## 9. REAGENT PREPARATION

Equilibrate all reagents and samples to room temperature (18 - 25°C) prior to use.

### 9.1 1X Biotinylated anti-mouse Angiopoietin-2

Biotinylated anti-mouse Angiopoietin-2 antibody must be diluted 1:100 with the Antibody Diluent Buffer and mixed thoroughly (i.e. add 1  $\mu$ L Biotinylated anti-mouse Angiopoietin antibody to 99  $\mu$ L Antibody Diluent Buffer.) The total volume required should be; 100  $\mu$ L/well multiplied by the total number of wells (allow 100  $\mu$ L - 200  $\mu$ L extra for pipetting error).

### 9.2 1X Avidin-Biotin-Peroxidase Complex

Avidin-Biotin-Peroxidase Complex (ABC) must be diluted 1:100 with ABC Diluent Buffer and mixed thoroughly (i.e. add 1  $\mu$ L ABC to 99  $\mu$ L ABC Diluent Buffer.) The total volume required should be; 100  $\mu$ L/well multiplied by the total number of wells (allow 100  $\mu$ L - 200  $\mu$ L extra for pipetting error).

### 9.3 0.01 M TBS

Add 1.2 g Tris, 8.5 g NaCl; 450  $\mu$ L of purified acetic acid or 700  $\mu$ L of concentrated hydrochloric acid to distilled water and adjust pH to 7.2 - 7.6. Finally, adjust the total volume to 1 L with distilled water.

### 9.4 0.01 M PBS

Add 8.5 g NaCl, 1.4 g Na<sub>2</sub>HPO<sub>4</sub> and 0.2 g NaH<sub>2</sub>PO<sub>4</sub> to distilled water and adjust pH to 7.2 - 7.6. Finally, adjust the total volume to 1 L with distilled water.

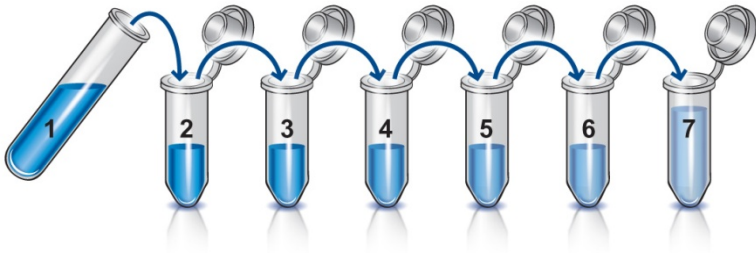
## 10. STANDARD PREPARATIONS

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use. Reconstitution of the mouse Angiotensin-2 standard should be prepared no more than 2 hours prior to the experiment. Two tubes of Angiotensin-2 standard (10 ng per tube) are included in each kit. Use one tube for each experiment.

- 10.1 Prepare a 10 ng/mL **Standard #1** by reconstituting the Angiotensin-2 standard with addition of 1 mL Sample Diluent Buffer. Hold at room temperature for 10 minutes. This 10 ng/mL **Standard #1** 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.
- 10.2 Label seven tubes with #2 - 8.
- 10.3 Add 300  $\mu$ L Sample Diluent Buffer into tubes #2 - 8.
- 10.4 Prepare **Standard #2** by transferring 300  $\mu$ L from Standard #1 to tube #2. Mix thoroughly and gently.
- 10.5 Prepare **Standard #3** by transferring 300  $\mu$ L from Standard #2 to tube #3. Mix thoroughly and gently.
- 10.6 Prepare **Standard #4** by transferring 300  $\mu$ L from Standard #3 to tube #4. Mix thoroughly and gently.
- 10.7 Using the table below as a guide, repeat for tubes #5 through #7.
- 10.8 **Standard #8** contains no protein and is the Blank control.

# ASSAY PREPARATION

Standard #	Sample to Dilute	Volume to Dilute ( $\mu\text{L}$ )	Volume of Diluent ( $\mu\text{L}$ )	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	<b>Step 10.1</b>				<b>10,000</b>
2	Standard #1	300	300	10,000	5,000
3	Standard #2	300	300	5,000	2,500
4	Standard #3	300	300	2,500	1,250
5	Standard #4	300	300	1,250	625
6	Standard #5	300	300	625	312
7	Standard #6	300	300	312	156
8	None	-	300	-	-



## **11. SAMPLE COLLECTION AND STORAGE**

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.

### **11.1 Cell Culture Supernatants**

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

### **11.2 Cell Lysates**

After sufficient splitting, there should be no obvious cell sediment. Centrifuge cell lysates at approximately 10,000 x *g* for 5 minutes. Collect the cell lysate supernatants to go ahead.

### **11.3 Serum**

Allow the serum to clot in a serum separator tube (about 4 hours) at room temperature. Centrifuge at approximately 1,000 x *g* for 15 minutes. Analyze the serum immediately or aliquot and store samples at -20°C.

### **11.4 Plasma**

Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1,000 x *g* within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C.

## 12. SAMPLE PREPARATION

### **General Sample information:**

The user needs to estimate the concentration of the target protein in the sample and select the correct dilution factor so that the diluted target protein concentration falls near the middle of the linear regime in the standard curve.

Dilute the samples using the provided Sample Diluent Buffer. The following is a guideline for sample dilution. Several trials may be necessary to determine the optimal dilution factor. The sample must be thoroughly mixed with the Sample Diluent Buffer before assaying.

- High target protein concentration (100 - 1000 ng/mL). The working dilution is 1:100. i.e. Add 1  $\mu$ L sample into 99  $\mu$ L Sample Diluent Buffer
- Medium target protein concentration (10 - 100 ng/mL). The working dilution is 1:10. i.e. Add 10  $\mu$ L sample into 90  $\mu$ L Sample Diluent Buffer
- Low target protein concentration (156 - 10,000 pg/mL). The working dilution is 1:2. i.e. Add 50  $\mu$ L sample to 50  $\mu$ L Sample Diluent Buffer
- Very Low target protein concentration ( $\leq$  156 pg/mL). No dilution necessary, or the working dilution is 1:2.

## **13. PLATE PREPARATION**

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents
- Unused well strips should be returned to the plate packet and stored at 4°C
- For each assay performed, a minimum of 2 wells must be used as blanks, omitting primary antibody from well additions
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates)
- Well effects have not been observed with this assay. Contents of each well can be recorded on the template sheet included in the Resources section

## **14. ASSAY PROCEDURE**

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**
  - 14.1 Prepare all reagents, working standards, and samples as directed in the previous sections
  - 14.2 Add 100  $\mu$ L of prepared standards and diluted samples to appropriate wells.
  - 14.3 Seal the plate with a new plate seal and incubate at 37°C for 90 minutes.
  - 14.4 Remove the seal, discard contents of each well, and blot the plate onto paper towels or other absorbent material. Do NOT let the wells completely dry at any time.
  - 14.5 Add 100  $\mu$ L of 1X Biotinylated anti-mouse Angiopoietin-2 antibody into each well and incubate the plate at 37°C for 60 minutes.
  - 14.6 Wash the plate three times with 300  $\mu$ L 0.01 M TBS or 0.01 M PBS, and each time let the washing buffer stay in the wells for one minute. Discard the washing buffer and blot the plate onto paper towels or other absorbent material.  
*Note:* For automated washing, aspirate all wells and wash THREE times with PBS or TBS buffer, overfilling wells with each wash. Blot the plate onto paper towels or other absorbent material.
  - 14.7 Add 100  $\mu$ L of 1X Avidin-Biotin-Peroxidase Complex working solution into each well and incubate the plate at 37°C for 30 minutes.
  - 14.8 Wash plate five times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1 - 2 minutes. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (See Step 14.6 for plate washing method).

- 14.9 Add 90  $\mu\text{L}$  of prepared TMB color developing agent into each well and incubate plate at 37°C in dark for 15 – 25 minutes.

*Note:* The optimal incubation time should be determined by end user. The shades of blue should be seen in the wells with the four most concentrated mouse Angiopoietin standard solutions; the other wells show no obvious color.

- 14.10 Add 100  $\mu\text{L}$  of prepared TMB Stop Solution into each well. The color changes into yellow immediately.
- 14.11 Read the O.D. absorbance at 450 nm in a microplate reader within 30 minutes after adding the stop solution.

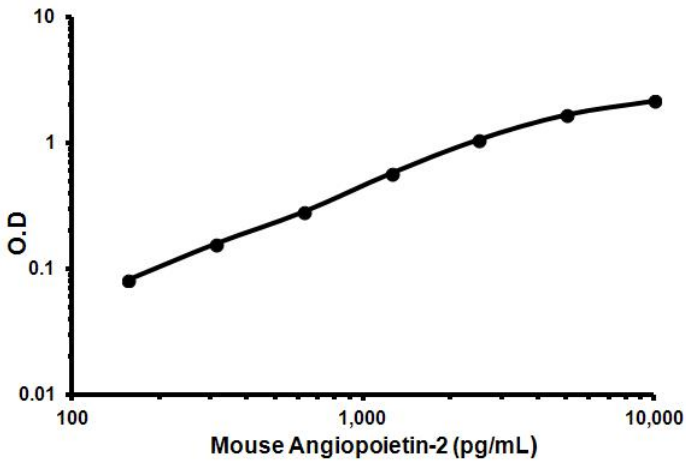
### 15. CALCULATIONS

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 mouse Angiopoietin-2 concentration of the samples can be interpolated from the standard curve.

Note: If the samples measured were diluted, make sure to account for this in your calculations.

## 16. TYPICAL DATA

**TYPICAL STANDARD CURVE** – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.



Conc. (pg/mL)	O.D. 450nm
0.0	0.009
156	0.082
312	0.159
625	0.285
1,250	0.578
2,500	1.073
5,000	1.689
10,000	2.174

### **17. TYPICAL SAMPLE VALUES**

**RANGE** – 156 - 10,000 pg/mL

**SENSITIVITY** – < 10 pg/mL

### **18. ASSAY SPECIFICITY**

This kit detects both endogenous and recombinant mouse Angiotensin II.

No detectable cross-reactivity with other relevant proteins.

## 19. TROUBLESHOOTING

<b>Problem</b>	<b>Cause</b>	<b>Solution</b>
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standards dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Samples give higher value than the highest standard	Starting sample concentration is too high.	Dilute the specimens and repeat the assay
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the kit	Store the all components as directed.

20. NOTES







**Technical Support**

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