

## HUMAN ANGIOPOIETIN-2 (ANGPT-2) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF  
HUMAN ANGPT-2 CONCENTRATIONS IN CELL  
CULTURE SUPERNATES, SERUM, AND PLASMA



ALWAYS REFER TO LOT SPECIFIC PROTOCOL  
PROVIDED WITH EACH KIT FOR  
INSTRUCTIONS. PROTOCOL MUST BE  
READ BEFORE USING THIS PRODUCT.

FOR RESEARCH USE ONLY. NOT FOR USE IN  
DIAGNOSTIC PROCEDURES.

### PRODUCT INFORMATION:

ELISA NAME	HUMAN ANGIOPOIETIN-2 (ANGPT-2) ELISA
Catalog No.	SK00066-01
Lot No.	
Formulation	96 T
Standard range	93.8 - 6000 pg/mL
Sensitivity	20 pg/mL
Sample Volume	100 µL
Dilution Factor	Optimal dilutions should be determined by each laboratory for each application
Sample Type	Serum, EDTA Plasma, Cell Culture Supernates
Specificity	Human ANGPT-2 only
Calibration	Human ANGPT-2 Recombinant
Intra-assay Precision	4 - 8%
Inter-assay Precision	8 - 12%
Storage	2 - 8° C
This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.	

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## DESCRIPTION

This Human ANGPT-2 ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human ANGPT-2 from cell culture supernates, serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human ANGPT-2 and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural ANGPT-2 samples.

## ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for human ANGPT-2. The capture antibody can bind to the human ANGPT-2 in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against human ANGPT-2 is added to the wells. After another washing of the plate, Streptavidin-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution (TMB) is added to the wells and color develops in direct proportion to the amount of human ANGPT-2 bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

## PROCEDURAL LIMITATIONS

\_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

\_This ELISA kit should not be used beyond the expiration date on the kit label.

\_Do not mix reagents with those from other lots or sources.

\_It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.

\_Each laboratory must determine the optimal dilution factors for the samples being assayed with a pretest. If samples generate values that are not within the dynamic range of the standard curve, further concentrate or dilute the samples as required with Dilution Buffer and repeat the assay.

\_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.

\_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

## COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
<b>ANGPT-2 Microplate</b> – 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against ANGPT-2.	<b>066-01-01</b>	<b>1 plate</b>
<b>ANGPT-2 Standard</b> – 12,000 pg/vial of recombinant human ANGPT-2 in a buffered protein base with preservative; lyophilized.	<b>066-01-02</b>	<b>1 vial</b>
<b>Detection Antibody Concentrate</b> – 1.05 mL/vial, 10-fold concentrate of biotinylated antibody against ANGPT-2 with preservative; lyophilized.	<b>066-01-03</b>	<b>1 vial</b>
<b>Positive Control</b> – one vial of recombinant ANGPT-2; lyophilized.	<b>066-01-04</b>	<b>1 vial</b>
<b>Streptavidin-HRP Conjugate</b> – 60 µL/vial, 200-fold concentrated solution of Streptavidin conjugate to HRP with preservative.	<b>SAHRP</b>	<b>1 vial</b>
<b>Dilution Buffer</b> – 60 mL of buffered protein based solution with preservative.	<b>DB01</b>	<b>1 bottle</b>
<b>Antibody Diluent Solution Concentrate</b> – 11 mL of buffered protein based solution with preservative; lyophilized.	<b>DB32</b>	<b>1 tube</b>
<b>Wash Buffer</b> – 50 mL of 10-fold concentrated buffered surfactant, with preservative.	<b>WB01</b>	<b>1 bottle</b>
<b>TMB Substrate Solution</b> – 11 mL of TMB substrate solution.	<b>TMB01</b>	<b>1 bottle</b>
<b>Stop Solution</b> – 11 mL of 0.5M HCl.	<b>S-STOP</b>	<b>1 bottle</b>
<b>Plate Sealer</b>	<b>EAPS</b>	<b>1</b>
<b>Plastic Pouch</b>	<b>P01</b>	<b>1</b>

## STORAGE

**Unopened Kit:** Store at 2 – 8° C for up to 8 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate should be stored at -20° C or -70° C. Do not use kit past expiration date.

**Opened / Reconstituted Reagents:** Reconstituted Standard (stock) solution and Detection Antibody concentrated solution SHOULD BE STORED at -20° C or -70° C for up to one month. Streptavidin-HRP Conjugate 200-fold concentrated solution and TMB Substrate Solution can be stored at 2 – 8° C for up to 8 months (**DO NOT FREEZE** and **PROTECT FROM LIGHT**). All other components may be stored at 2 – 8° C for up to 8 months.

**Microplate Wells:** Return unused strips to the plastic pouch with the desiccant pack. Microplate may be stored for up to 6 months at 2 – 8° C after opening.

### ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (250 – 300 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

### PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

### SAMPLE COLLECTION AND STORAGE

**Cell Culture Supernates** - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

**Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 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, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

**Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.**

### SAMPLE PREPARATION

Plasma and serum samples may not need to be diluted. **Optimal dilutions should be determined by each laboratory for each application.** Use polypropylene test tubes.

### REAGENT PREPARATION

**Bring all reagents to room temperature before use.**

**Wash Buffer** - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of 1x Wash Buffer.

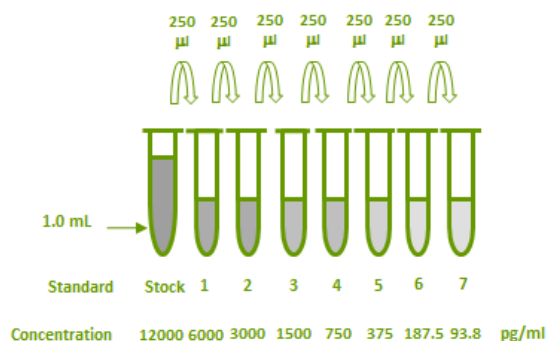
### Antibody Diluent Solution Concentrate –

Reconstitute the Antibody Diluent Solution Concentrate with 11.0 mL of Dilution Buffer in provided 15 mL centrifuge tube to prepare Antibody Diluent Solution.

### ANGPT-2 Standard - Refer to vial label for reconstitution volume.

Reconstitute the ANGPT-2 standard with 1 mL of Dilution Buffer. This reconstitution produces a stock solution of 12,000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of Dilution Buffer into tubes #1 to #7. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **6000 pg/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1.0 ml	12,000 pg/ml
# 1	250µl of stock	250µl	6000 pg/ml
# 2	250µl of 1	250µl	3000 pg/ml
# 3	250µl of 2	250µl	1500 pg/ml
# 4	250µl of 3	250µl	750 pg/ml
# 5	250µl of 4	250µl	375 pg/ml
# 6	250µl of 5	250µl	187.5 pg/ml
# 7	250µl of 6	250µl	93.8 pg/ml



**Detection Antibody** - Reconstitute the Detection Antibody Concentrate with 1.05 mL of **Antibody Diluent Solution (DB32)** to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of Antibody Diluent Solution (DB32) into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution. **Note:** Prepare 1 – 2 hours prior to use.

**Streptavidin-HRP Conjugate** - Transfer 60 µL of 200-fold concentrated stock solution to 11.94 mL of Dilution Buffer to prepare working solution. **Note:** 1X working solution of Streptavidin-HRP Conjugate should be used within a few days (**protect from light**).

**Positive Control** - Reconstitute the positive control with 1 mL of Dilution Buffer to make positive control working solution. **Note:** Positive control working solution could be reused within a few days if stored at -20° C to -70° C.

## ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the plastic pouch with the desiccant pack.
3. Add 100 µL per well of Dilution Buffer to Blank wells.
4. Add 100 µL of Standard solutions #7-#1 in reverse order of serial dilution, sample, or positive control per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with 1x Wash Buffer (300 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 Detection Antibody working solution to each well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Streptavidin-HRP Conjugate working solution to each well. Incubate for 60 minutes on microplate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of Substrate Solution to each well. Incubate for 2-5 minutes on microplate shaker at room temperature. **Protect from light.**
11. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 15 minutes, using a microplate reader set to 450 nm.

### CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control and samples, and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a log-log 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 ANGPT-2 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 STANDARD CURVE

This standard curve is for demonstration only. A new standard curve should be made for each set of samples assayed.

ANGPT-2 (PG/ML)	CORRECTED O.D. (450NM)
Blank	0 (0.112)
93.8	0.035
187.5	0.074
375	0.137
750	0.270
1500	0.527
3000	1.083
6000	1.849

- Lot No.:
- Positive Control :

### SPECIFICITY

PROTEINS	CROSS-REACTIVITY (%)
Human ANGPT-2	100%
Human ANGPT-1	0
Human Tie-1	0
Human Tie-2	0
Human TNF- $\alpha$	0
Mouse ANGPT-3	0
Mouse Tie-2	0

### SUMMARY OF ASSAY PROCEDURE

#### PREPARE REAGENTS, SAMPLES AND STANDARDS

Add 100  $\mu$ l of standard, samples, positive control to each well. Incubate for 2 hours on the plate shaker at RT. **Prepare Detection Antibody working solution 1-2 hours prior to use.**

Aspirate and wash 4 times.

Add 100  $\mu$ l Detection Antibody working solution to each well. Incubate for 2 hours on the plate shaker at RT.

Aspirate and wash 4 times.

Add 100  $\mu$ l Streptavidin-HRP conjugate working solution to each well. Incubate 60 min on plate shaker at RT. **Protect from light.**

Aspirate and wash 4 times.

Add 100  $\mu$ l Substrate Solution to each well. Incubate 2-5 min on the plate shaker at RT. **Protect from light.**

Add 100  $\mu$ l Stop Solution to each well. Read 450nm within 15 min.