

HUMAN ANGIOPOIETIN-2 (ANGPT-2) ELISA KIT

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



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

PURCHASE 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
Intra-assay Precision	4 - 8%
Inter-assay Precision	8 - 12%
Storage	2 - 8°C

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INTRODUCTION

Human ANGPT-2 immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure human ANGPT-2 in cell culture supernates, serum, and EDTA plasma. It contains recombinant human ANGPT-2 and antibodies raised against this protein. It has been shown to accurately quantify recombinant human ANGPT-2. Results obtained with naturally occurring ANGPT-2 samples showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that the immunoassay kit can be used to determine relative mass values for natural human ANGPT-2.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for ANGPT-2 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any ANGPT-2 present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated monoclonal antibody specific for ANGPT-2 is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, HRP link Streptavidin is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of ANGPT-2 bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

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_ 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.

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

_ If samples generate values higher than the highest standard, dilute the samples with dilution buffer and repeat the assay.

_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors

have been tested in the immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
ANGPT-2 Microplate – 96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse monoclonal antibody against ANGPT-2.	066-01-01	1 plate
ANGPT-2 Standard – 12,000 pg/vial of recombinant human ANGPT-2 in a buffered protein base with preservatives; lyophilized.	066-01-02	1 vial
Detection Antibody Concentrate – 105 µL/vial, 100-fold concentrated of biotinylated monoclonal antibody against ANGPT-2 with preservatives; lyophilized.	066-01-03	1 vial
Positive Control – one vial of recombinant ANGPT-2, lyophilized	066-01-04	1 vial
Streptavidin-HRP Conjugate – 60 µl/vial, 200-fold concentrated solution of Streptavidin conjugate to HRP with preservatives	SAHRP	1 vial
Dilution Buffer – 60 mL of buffered protein based solution with preservatives	DB01	1 bottle
Antibody Diluent Solution Concentrate – 11 mL of buffered protein based solution with preservatives	DB32	1 tube
Wash Buffer – 50 mL of 10-fold concentrated buffered surfactant, with preservative.	WB01	1 bottle
TMB Substrate Solution – 11 mL of TMB substrate solution	TMB01	1 bottle
Stop Solution – 11 mL of 0.5M HCl	S-STOP	1 bottle
Plate Sealer	EAPS	1
Plastic Pouch	P01	1

STORAGE

Unopened Kit: Store at 2 - 8° C for up to 12 months.

For longer storage, unopened Standard, Positive Control, Detection Antibody Concentrate and Antibody Diluent Solution Concentrate should be stored at -20°C or -70°C. Do not use kit past expiration date.

Opened / Reconstituted Reagents: Reconstituted Detection Antibody Solution and Antibody Diluent Solution SHOULD BE STORED at -20°C or -70°C for up to one month. DO NOT FREEZE RECONSTITUTED STANDARD, store at 2-8°C up to one month. Streptavidin-HRP Conjugate 200-fold Concentrate and other components may be stored at 2 - 8°C for up to 12 months.

Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack, reseal along entire edge of zip-seal. Microplate may be stored for up to 6 months at 2 - 8°C after opening.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with correction wavelength set at 540 nm or 570 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

PRECAUTIONS FOR USE

All reagents should be considered as potentially hazardous. The stop solution contains diluted hydrochloric acid. Appropriate care, therefore, should be taken while handling this solution. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.

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.

Note: Use Aprotinin (enzyme inhibitor) (Code No.: 00700-01-25) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

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.

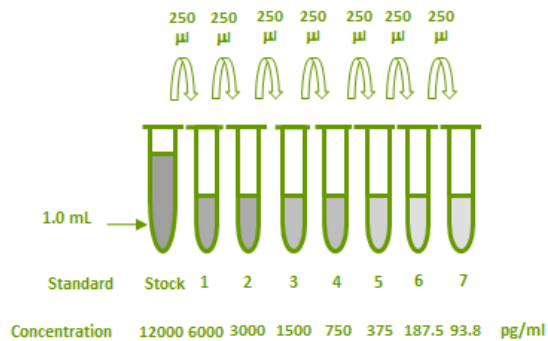
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of Wash Buffer.

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 1.0 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** with 105 µL of Antibody Diluent Solution to produce a 100-fold concentrated stock solution. Pipette 10.395 mL of Antibody Diluent Solution into a 15 mL centrifuge tube and transfer 105 µL of 100-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.

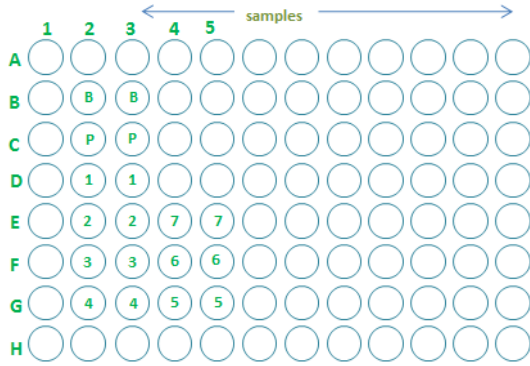
Positive Control - Reconstitute the positive control with 1.0 mL of Dilution Buffer to make positive control working solution. **Note:** Positive control working solution should be used within a few days.

ASSAY PROCEDURE

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

1. Prepare all reagents and working standards as directed in the previous sections.

- Remove excess micro-plate strips from the plate frame, return them to the plastic pouch with the desiccant pack, reseal.
- Add 100 µL of Dilution Buffer to Blank wells (B2, B3).
- Add 100 µL of Standard (D2, D3 to G2, G3 and E4, E5 to G4, G5), sample, or positive control (C2, C3) per well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature. A plate layout is provided to record standards and samples assayed.
- 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.
- Add 100 µL of Detection Antibody working solution to each well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature.
- Repeat the aspiration/wash as in step 5.
- Add 100 µL of Streptavidin-HRP Conjugate working solution to each well. Incubate for 60 minutes on micro-plate shaker at room temperature. **Protect from light.**
- Repeat the aspiration/wash as in step 5.
- Add 100 µL of Substrate Solution to each well. Incubate for 3-7 minutes at room temperature. **Protect from light.**
- 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.
- Determine the optical density of each well within 15 minutes, using a micro-plate 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 DATA

A standard curve should be generated for each set of samples assayed.

ANGPT-2 (PG/ML)	CORRECTED O.D. (450NM)
Blank	0 (0.120)
93.8	0.067
187.5	0.099
375	0.164
750	0.287
1500	0.541
3000	0.941
6000	1.850

- Lot No.:
- Positive Control : 800 - 1700 pg/mL

CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant human ANGPT-2.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of ANGPT-2 was 20 pg/mL.

SPECIFICITY

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

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS

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Add 100 µl of standard, samples, positive control to each well. Incubate for 2 hours at RT. **Prepare Detection Antibody working solution 1-2 hours prior to use.**

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Aspirate and wash 4 times.

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

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Aspirate and wash 4 times.

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

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Aspirate and wash 4 times.

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Add 100 µl Substrate Solution to each well. Incubate 3-7 min on the plate shaker. **Protect from light.**

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Add 100 µl Stop Solution to each well. Read 450nm within 15 min