HUMAN ANGIOPOIETIN-1 (ANGPT-1) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN ANGIOPOIETIN-1 CONCENTRATIONS IN CELL CULTURE SUPERNATES, SERUM, AND EDTA PLASMA

ALWAYS REFER TO LOT SPECIFIC PROTCOL 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-1 (ANGPT-1) ELISA
Catalog No.	SK00631-01
Lot No.	
Formulation	96 T
Standard range	156 – 10,000 pg/mL
Sensitivity	78 pg/mL
Sample Volume	100 µl
Sample Type	Serum, Plasma, Cell Culture sipernates
Dilution Factor	**8 (Optimal dilutions should be determined by each laboratory for each application)**
Specificity	Human ANGIOPOIETIN-1
Calibration	Human ANGIOPOIETIN-1 Recombinant
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 10%
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 Angiopoietin-1 ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human Angiopoietin-1 from cell culture supernates, serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human Angiopoietin-1 and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural Angiopoietin-1 samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for human Angiopoietin-1. The capture antibody can bind to the human Angiopoietin-1 in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against human Angiopoietin-1 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 Angiopoietin-1 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
Angiopoietin-1 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against Angiopoietin-1.	**631-01-01**	**1 plate**
Angiopoietin-1 Standard – 10,000 pg/vial of recombinant human Angiopoietin-1 in a buffered protein base with preservatives; lyophilized.	**631-01-02**	**1 vial**
Detection Antibody Concentrate – 1.05 mL/vial, 10-fold concentrated of biotinylated polyclonal antibody against Angiopoietin-1 with preservatives; lyophilized.	**631-01-03**	**1 vial**
Positive Control - one vial of recombinant human Angiopoietin-1, lyophilized	**631-01-04**	**1 vial**
Streptavidin-HRP Conjugate - 60 µl/vial, 200-fold concentrated solution of Streptavidin conjugate to HRP	**SAHRP**	**1 vial**
Dilution Buffer - 60mL of buffered protein based solution with preservatives	**DB01**	**1 bottle**
Antibody Diluent Solution Concentrate – 11 mL of buffered protein based solution with preservatives	**DB20**	**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 solution	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1 piece**
Plastic Pouch	**P01**	**1 piece**

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 - 70° 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 -70° 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

Serum and plasma samples may require an 8-fold dilution. A suggested 8-fold dilution is 30 µL sample + 210 µL Dilution Buffer.
Optimal dilutions should be determined by each laboratory for each application.
Use polypropylene test tubes.

REAGENT PREPARATION

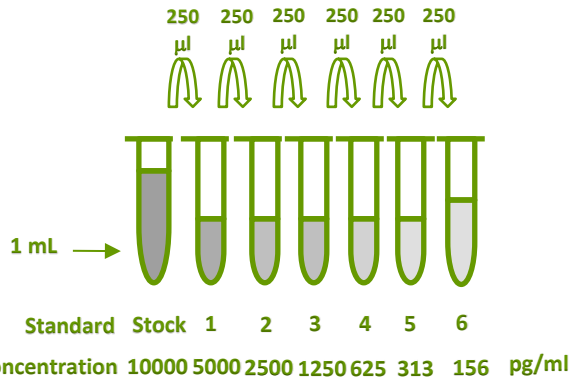
Bring all reagents to room temperature before use.

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.

Angiopoietin-1 Standard - Refer to vial label for reconstitution volume. Reconstitute the **Angiopoietin-1** standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 10,000 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 #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 10,000 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	10,000 pg/ml
# 1	250µl of stock	250µl	5000 pg/ml
# 2	250µl of 1	250µl	2500 pg/ml
# 3	250µl of 2	250µl	1250 pg/ml
# 4	250µl of 3	250µl	625 pg/ml
# 5	250µl of 4	250µl	313 pg/ml
# 6	250µl of 5	250µl	156 pg/ml



Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with 1.05 mL of **Antibody Diluent Solution** to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of Antibody Diluent Solution 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 - Pipette 11.94 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 60 µL of 200-fold concentrated stock solution to prepare working solution. **Note:** 1x working solution of Streptavidin HRP Conjugate should be used within a few days **(protect from light, do not freeze)**.

Positive Control - Reconstitute the Positive Control with 1.0 mL of Dilution Buffer. **Note:** Positive Control could be reused within a few days if stored at -20 °C or -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 micro-plate strips from the plate frame, return them to the plastic pouch with the desiccant pack, reseal.
3. Add 100 µL per well of Dilution Buffer to Blank wells.
4. Add 100 µL of Standard Dilutions, sample, or positive control per well. Cover with plate sealer. Incubate for 2 hours on micro-plate 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 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 micro-plate 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 micro-plate 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 5-10 minutes on micro-plate 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. Read plate using a microplate reader set to 450 nm within 15 minutes.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control and sample, 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 Angiopoietin-1 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 provided for demonstration only. A new standard curve should be generated for each set of samples assayed.

STANDARD (PG/ML)	AVERAGE OD450NM (CORRECTED)
Blank	0 (0.09)
156	0.029
313	0.063
625	0.123
1250	0.242
2500	0.512
5000	1.076
10,000	1.980

- Lot No.:
- Positive Control:

SPECIFICITY

This assay recognizes both natural and recombinant human Angiopoietin-1. The factors listed below were prepared at 500 ng/mL in Dilution Buffer, and assayed for cross reactivity

PROTEINS	CROSS-REACTIVITY (%)
Human Angiopoietin-1	100
Human Angiopoietin-2	0
Human Tie-2	0
Human Tie-1	0
Human ANGPTL-4	0
Human ANGPTL-3	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100µl of standard, samples, positive control to each well. Incubate 2 hours on the plate shaker at RT. **Note:** Prepare Detection Antibody working solution 1-2 hours prior to use.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Detection Antibody working solution to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Streptavidin HRP conjugate working solution to each well. Incubate 60 min on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate 5-10 min on the plate shaker. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read 450 nm within 15 min