

ELISA Kit Components	Amount	Cat/Part No.
Anti-Human ANG-2 Microwell Plate	8-well strips (12)	100-181
Human ANG-2 Positive Control	0.65 ml	100-182
Human ANG-2 Standard 1 ng/ml	0.65 ml	100-183B
Human ANG-2 Standard 2 ng/ml	0.65 ml	100-183C
Human ANG-2 Standard 4 ng/ml	0.65 ml	100-183D
Human ANG-2 Standard 8 ng/ml	0.65 ml	100-183E
Human ANG-2 Standard 16 ng/ml	0.65 ml	100-183F
Anti-Human ANG-2 Detection Ab (100X)	0.15 ml	100-184
Streptavidin HRP Conjugate (100X)	0.15 ml	S-HRP100
Sample Diluent Concentrate (20X)	10 ml	SD-20T
Wash Solution Concentrate (100X)	10 ml	WB-100
TMB Substrate	12 ml	80091
Stop Solution	12 ml	80101
Product Manual	1 ea	M-100-180-APH

Instruction Manual No. M-100-180-APH

Human ANG-2

ELISA Kit Cat. No. 100-180-APH

For Quantitative Determination of Human Angiopietin-2 [ANG-2] in Solution

Other ELISA kits available from ADI

Mouse: Albumin, IgA, IgG, IgG1, IgG2a, IgG3, IgG2b, IgM, Leptin, Acrp30, CRP, Haptoglobin, VEGF, SAP.

Human: BD-1, BD-2, BD-3, NP-1 **and:** Adiponectin (Acrp30 and gAcrp30), Albumin, Aldosterone, AFP, Angiogenin, Angiopietin-2, beta-2M, C-peptide, CRP, Cox-2, Ferritin, PSA, fPSA, GH, IgG, IgM, IgA, Insulin, NSE, CA125, CA199, CA242, PAP, SHBG, LH, FSH, TSH, T3, T4, and Steroid ELISA kits (cortisol, E2, testosterone, progesterone etc).

Rat: Albumin, CRP, IgG, IgM, Alpha 1 Acid glycoprotein

Bovine: Albumin, IgG, IgM, Lactoferrin, Transferrin

Monkey: IgM, IgG, IgA, CRP, IgE

Chicken: IgY(G), IgM, Ovalbumin

Rabbit: CRP, IgG

Pig: Albumin, IgG, IgM

Dog: CRP, IgG, IgM

Cat: IgG, IgM

Goat: IgG



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DRAFT MANUAL: PLEASE CONSULT THE MANUAL SUPPLIED WITH THE KIT FOR ANY LOT SPECIFIC CHANGES.

INTENDED USE

The Human ANG-2 ELISA Kit is an in vitro immunoassay for research use in the quantification of Human ANG-2 in cultures of human cells and in appropriately qualified samples from serum, saliva, or other tissue fluids.

INTRODUCTION

Angiogenesis accounts for the formation of vasculature into previously avascular organs such as brain and kidney. Angiogenic activity in the adult is required during the normal tissue repair, and for the remodeling of the female reproductive organs (ovulation and placental development). Certain pathological conditions, such as tumor growth and diabetic retinopathy, also require angiogenesis.

The angiopoietin (Ang) family of growth factors includes four members, all of which bind to the endothelial receptor tyrosine kinase Tie2. Two of the Angs, Ang-1 and Ang-4, activate the Tie2 receptor, whereas Ang-2 and Ang-3 inhibit Ang-1-induced Tie2 phosphorylation.

Angiopoietin-1 (Ang-1) is a secreted growth factor, which binds to and activates the Tie-2 receptor tyrosine kinase. This growth factor enhances endothelial cell survival and capillary morphogenesis, and also limits capillary permeability. Ang-2 binds the same receptor but does not activate it. Ang-2 may act as an antagonist for Ang-1 and Tie-2. Ang-2 disrupts capillary integrity, increasing vessel growth when vascular endothelial growth factor (VEGF) levels are high, but causing vessel regression when VEGF levels are low. Angiopoietin and Tie genes are expressed in the metanephros, the precursor of the adult kidney, where they may play a role in endothelial precursor growth/development.

PRINCIPLE OF THE TEST

The Human ANG-2 ELISA kit is based on the binding of human ANG-2 in samples to two antibodies, one immobilized on the microtiter wells, and the other conjugated to biotin, which then binds to a streptavidin horseradish peroxidase (HRP) conjugate. After a washing step, chromogenic substrate is added and color is developed by the enzymatic reaction of HRP on the TMB substrate, which is directly proportional to the amount of ANG-2 present in the sample. A Stop Solution is added to terminate the reaction, and absorbance at 450nm is then measured using an ELISA microtiter well reader. The concentration of ANG-2 in samples is calculated from a standard curve of purified recombinant human ANG-2 of designated concentration.

STORAGE AND STABILITY

The microtiter well plate and all other reagents, if unopened, are stable at 2-8°C until the expiration date printed on the kit box label. Stabilities of the working solutions are indicated under Reagent Preparation.

PERFORMANCE CHARACTERISTICS & EXPECTED RESULTS

Specificity

The antibodies used in this kit have been affinity purified using a purified recombinant human ANG-2 immunosorbent and have been shown by ELISA to react specifically with human ANG-2, and to have essentially no reactivity with the following human proteins: ANG-1, Tie-1 & 2, TNF α , VEGF and FGF.

Lab Notes

CALCULATION OF RESULTS

The results may be calculated using any immunoassay software package. The four-parameter curve-fit is recommended. If software is not available, Human ANG-2 concentrations may be determined as follows:

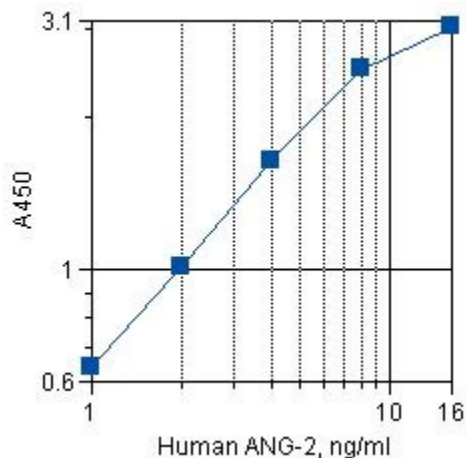
1. Calculate the mean OD of duplicate samples.
2. On graph paper plot the mean OD of the standards (y-axis) against the concentration (ng/ml) of Human ANG-2 (x-axis). Draw the best fit curve through these points to construct the standard curve. A point-to-point construction is most common and reliable.
3. The Human ANG-2 concentrations in unknown samples and controls can be determined by interpolation from the standard curve.
4. Multiply the values obtained for the samples by the dilution factor of each sample.
5. Samples producing signals higher than the 16 ng/ml standard should be further diluted and re-assayed.

TYPICAL RESULTS

The following data are for illustration purposes only. A complete standard curve should be run in every assay to determine sample values.

Wells	Standards, Control & Samples	A450 nm mean	ANG-2 ng/ml
1A, B	Negative Diluent Control	0.29	0
1C, D	1 ng/ml Standard	0.64	1
1E, F	2 ng/ml Standard	1.11	2
1G, H	4 ng/ml Standard	1.54	4
2A, B	8 ng/ml Standard	2.50	8
2C, D	16 ng/ml Standard	3.02	16
2G, H	Positive Serum Control [Value: 3.9 – 9.5 ng/ml]	2.07	5.6
3A, B	Sample [Diluted 1:5] Calculated: 5-fold dilution x 2.7 ng/ml = 13.5 ng/ml in medium	1.30	2.7

A typical assay Standard Curve (do not use for calculating sample values)



KIT CONTENTS

Ready For Use: Store as indicated on labels.

Component	Part #	Amt	Contents
Anti-Human ANG-2 Microwell Strip Plate	100-181	8-well strips (12)	Coated with purified anti-human ANG-2 antibodies. Return unused strips to the pouch with desiccant; re-seal and store refrigerated.
Positive Control [ANG-2] range on label	100-182	0.65 ml	ANG-2 with stated concentration range; diluted in buffer with protein, detergents and ProClin 300 as stabilizers.
Human ANG-2 Standards			
1 ng/ml	100-183B	0.65 ml	Five (5) vials, each containing the specified concentration of recombinant ANG-2 ; diluted in buffer with protein, detergents and ProClin 300 as stabilizers.
2 ng/ml	100-183C	0.65 ml	
4 ng/ml	100-183D	0.65 ml	
8 ng/ml	100-183E	0.65 ml	
16 ng/ml	100-183F	0.65 ml	
TMB Substrate	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
Stop Solution	80101	12 ml	1% sulfuric acid.

To Be Reconstituted: Store as indicated.

Component	Instructions for Use
Sample Diluent Concentrate (20x) Cat. No. SD-20T, 10ml	Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as Working Sample Diluent and store at 2-8°C until the kit lot expires or is used up.
Anti-Human ANG-2 Detection Antibody Concentrate (100x) Part No. 100-184, 0.15ml	Biotinylated anti-human ANG-2 in buffer with protein, detergents and ProClin 300 as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of Working Sample Diluent is sufficient for 1 8-well strip. Use within the working day and discard. Return concentrate to 2-8°C storage.
Streptavidin-HRP Conjugate Concentrate (100x) Part No. S-HRP100, 0.15ml	Peroxidase conjugated streptavidin antibody in buffer with protein, detergents and ProClin 300 as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of Working Sample Diluent is sufficient for 1 8-well strip. Use within the working day and discard. Return concentrate to 2-8°C storage.
Wash Solution Concentrate (100x) Cat. No. WB-100, 10ml	Dilute the entire volume, 10ml, to 1L with distilled or deionized water into a clean stock bottle. Label as Working Wash Solution and store at ambient temperature until kit is used entirely.

Materials Required But Not Provided:

- Pipettors and pipettes that deliver 100ul and 1-10ml. A multi-channel pipettor is recommended.
- Disposable glass or plastic 5-15ml tubes for diluting samples, Detection Antibody Concentrate and Streptavidin-HRP Concentrate.
- Graduated cylinder to dilute Wash Concentrate and Sample Diluent concentrate; 200ml to 1L.
- Stock bottle to store diluted Wash Solution; 200ml to 1L.
- Distilled or deionized water to dilute reagent concentrates.
- Microwell plate reader at 450 nm wavelength.

PRECAUTIONS AND SAFETY INSTRUCTIONS

Standards, Controls, Sample Diluent, and Detection Antibody contain ProClin 300 (0.05%, v/v). Stop Solution contains 1% sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water.

MSDS for TMB, sulfuric acid and ProClin 300, if not already on file, can be requested or obtained from the ADI website.

SPECIMEN COLLECTION AND HANDLING

Culture medium, serum and other biological fluids may be used as samples with proper dilution to avoid solution matrix interference.

For **serum**, collect blood by venipuncture, allow clotting, then separate the serum by centrifugation at room temperature.

For **other samples**, including tissue culture media, clarify the sample by centrifugation and/or filtration prior to dilution in Working Sample Diluent. If samples will not be assayed immediately, store refrigerated for up to a week, or frozen for long-term storage. Avoid freeze-thaw cycles.

QUALITY CONTROL

Sample Controls A Positive Control is provided with the kit, assigned with an ANG-2 concentration value range. Recovery in this range is an indicator of proper assay performance. Each lab should also assay internal control samples, which represent the lab's expected sample population and that are maintained stabilized. A Negative Diluent Control should also be run.

Technique Accurate and reproducible assay results rely on good lab technique regarding pipetting, plate washing and handling of samples and reagents.

Equipment Precision of results relies on uniform and effective washing techniques; an automatic washer may be used. ELISA reader and pipettes should be properly calibrated.

ASSAY PROCEDURE

Bring all reagents to room temperature (18-30° C) equilibration (at least 30 minutes).

DILUTE Samples at least 5-fold in Working Sample Diluent, e.g., 100ul sample + 400ul Diluent.

DO NOT dilute the Standards or Positive Control Serum.

PERFORMED ALL STEPS AT ROOM TEMPERATURE. After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation.

1. Set-up

- Determine the number of wells for the assay run. Duplicates are recommended, to include 10 Standard wells and 2 wells for each sample and control to be assayed.
- Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.
- Prior to sample addition, add 200-300ul Working Wash Solution to each well and let stand about 5 minutes.
- Aspirate or dump the liquid and pat the plate dry on a paper towel.

2. 1st Incubation

[100ul – 60 min; 4 washes]

- Add 100ul of standards, samples and controls each to pre-determined wells.
- Tap the plate gently to mix reagents and incubate for 60 minutes.
- Wash wells 4 times and pat dry on fresh paper towels. An automatic plate washer may be used. Improper washes may lead to falsely elevated signals and poor reproducibility.

3. 2nd Incubation

[100ul – 60 min; 4 washes]

- Add 100ul of Working Detection Antibody to each well.
- Incubate for 60 minutes.
- Wash wells 4 times as in step 2.

4. 3rd Incubation

[100ul – 30 min; 5 washes]

- Add 100ul of Working Streptavidin-HRP Conjugate to each well.
- Incubate for 30 minutes.
- Wash wells 5 times as in step 2.

5. Substrate Incubation

[100ul – 15 min]

- Add 100ul TMB Substrate to each well. The liquid in the wells will begin to turn blue.
- Incubate for 15 minutes in the dark, e.g., place in a drawer or closet.

Note: If your microplate reader does not register optical density (OD) above 2.0, incubate for less time, or read OD at 405-410 nm (results are valid).

6. Stop Step

[Stop: 100ul]

- Add 100ul of Stop Solution to each well.
- Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.

7. Absorbance Reading

- Use any commercially available microplate reader capable of reading at 450nm wavelength. Use a program suitable for obtaining OD readings, and data calculations if available.
- Read absorbance of the entire plate at 450nm using a single wavelength within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.