

ELISA kits available from ADI (see details at the web site)

#0010	Human Leptin		
#200-120-AGH	Human globular Adiponectin (gAcrp30)		
#0700	Human Sex Hormone Binding Glob (SHBG)		
#0900	Human IGF-Binding Protein 1 (IGFBP1)		
#1000	Human C-Reactive Protein (CRP)		
#100-110-RSH	Human Resistin /FIZZ3		
#100-140-ADH	Human Adiponectin (Acrp30)		
#100-160-ANH	Human Angiogenin		
#100-180-APH	Human Angiopoietin-2 (Ang-2)		
#100-190-B7H	Human Bone Morphogenic Protein 7 (BMP-7)		
#1190	Human Serum Albumin	#1200	Human Albumin (Urinary)
#1750	Human IgG (total)	#1760	Human IgM
#1800	Human IgE	#1810	Human Ferritin
#1210	Human Transferrin (Tf)		
#0020	Beta-2 microglobulin		
#1600	Human Growth Hormone (GH)		
#0060	Human Pancreatic Colorectal cancer (CA-242)		
#1820	Human Ovarian Cancer (CA125)	#1830	Human CA153
#1840	Human Pancreatic & GI Cancer (CA199)		
#1310	Human Pancreatic Lipase		
#1400	Human Prostatic Acid Phosphatase (PAP)		
#1500	Human Prostate Specific Antigen (PSA)	#1510	free PSA (fPSA)
#0500	Human Alpha Fetoprotein (AFP)		
#0050	Human Neuron Specific Enolase (NSE)		
#0030	Human Insulin	#0040	Human C-peptide
#0100	Human Luteinizing Hormone (LH)		
#0200	Human Follicle Stimulating Hormone (FSH)		
#0300	Human Prolactin (PRL)		
#0400	Human Chorionic Gonadotropin (HCG)	#0410	HCG-free beta
#0600	Human Thyroid Stimulating Hormone (TSH)		
#1100	Human Total Thyroxine (T4)	#1110	Human Free T4 (fT4)
#1650	Human free triiodothyronine (fT3)	#1700	Human T3 (total)
#1850	Human Cortisol	#1860	Human Progesterone
#1865	Human Pregnenolone	#1875	Human Aldosterone
#1880	Human Testosterone	#1885	Human free Testosterone
#1910	Human Androstenedione	#1920	Human Estradiol
#1925	Human Estrone	#1940	Dihydrotestosterone (DHT)
#1950	Human DHEA-sulphate (DHEA-S)		
#3400	Human serum Neopterin		
#3000	Human Rheumatoid Factors IgM (RF)		
#3100	Human anti-dsDNA		
#3200	Anti-Nuclear Antibodies (ANA)		

Instruction Manual No. M- 100-180-APH

Human Angiopoietin-2 (Ang-2)

ELISA Kit Cat # 100-180-APH, 96 Tests

For Quantitative Determination of ANG2 in serum, plasma, tissue homogenates and other biological fluids.



For In Vitro Research Use Only



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Angiopietin-2 (Ang-2) ELISA KIT # 100-180-APH Kit Contents (96 tests):

Components	#
Anti- ANG2 antibody coated microwell strips (96 wells, 12 x 8 wells), #100-180P	1 Plate
Standard Lyophilized , #100-181	2 vials
Sample / Standard dilution buffer , #100-182, 20 ml	1 bottle
Biotin-detection antibody (Concentrated), #100-183, 120 ul	1 vial
Antibody dilution buffer , #100-184, 10 ml	1 bottle
HRP-Streptavidin Conjugate , #100-185, 120 ul	1 vial
HRP-Streptavidin Conjugate dilution buffer , #100-186, 10 ml	1 bottle
TMB Substrate , #100180-TM, 10 ml	1 bottle
Stop Solution , #100180-ST, 10 ml	1 bottle
Wash buffer (25X) , #100180-WB, 30 ml	1 bottle
Complete Instruction Manual , M-100-180-APH	1

Intended Use

ADI's Human Angiopietin-2 (Ang-2) ELISA kit is a highly sensitive sandwich type assay for the quantitative measurement of ANG2 in serum, plasma, tissue homogenates and other biological fluids.. **For research use only (RUO), not for diagnostic procedures.**

General Information

Angiopietin-2 is also known as ANGPT2, AGPT2, ANG2, and is a secreted glycoprotein that plays a complex role in angiogenesis and inflammation. Ang2 is widely expressed during development, but it is restricted postnatally to highly angiogenic tissues such as the placenta, ovaries, and uterus. It is particularly abundant in vascular endothelial cells (EC) where it is stored in intracellular Weibel Palade bodies. Both Ang2 and the related Angiopietin1 (Ang1) are ligands for the receptor tyrosine kinase Tie 2. Ang2 functions as a proangiogenic factor, although it can also induce EC death and vessel regression. Upon its release from quiescent EC, it regulates vascular remodeling by promoting EC survival, proliferation, and migration and destabilizing the interaction between EC and perivascular cells. Ang2 is required for postnatal vascular remodeling, and it cooperates with Ang1 during lymphatic vessel development. It mediates the upregulation of ICAM1 and VCAM1 on EC, which facilitates the adhesion of leukocytes during inflammation. Ang2 competitively inhibit Ang1-induced endothelial cell responses mediated by Tie2, and reduces vascular integrity. But the role of Ang2 is controversial since the opposite outcomes has been reported in other studies. Over-expression of Ang2 disrupts the vascular remodeling, induce endothelial cell apoptosis, and may play an important regulating role in tumor angiogenesis.

For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.

3. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, ADI shall have no liability.

4. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

PERFORMANCE CHARACTERISTICS

Precision

The within and between assay precision of the Ang-2 ELISA Test System were determined by analyses on three 3 samples with low, middle and high level Ang-2.

CV (%) = SD/meanX100
 Intra-Assay: CV<8%
 Inter-Assay: CV<10%

Sensitivity

The Ang-2 ELISA Test System has a sensitivity of < 28.125 pg/ml.

Detection Range

46.875 - 3000pg/ml

Recovery

Matrices listed below were spiked with certain level of Ang-2 and the recovery rates were calculated by comparing measured value to the expected amount of Ang-2 in samples.

Matrix	Recovery range (%)	Average (%)
Serum (n=5)	90-105	95
EDTA plasma (n=5)	85-101	94
Heparin plasma (n=5)	86-105	96

Linearity

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of Ang-2 and their serial dilutions. The results were demonstrated by the percentage of calculated concentration to the expected.

Sample	1:2	1:4	1:8	1:16
Serum (n=5)	85-100%	86-101%	86-102%	90-105%
EDTA plasma (n=5)	84-93%	83-101%	86-100%	84-100%
Heparin plasma (n=5)	87-99%	85-96%	87-98%	83-97%

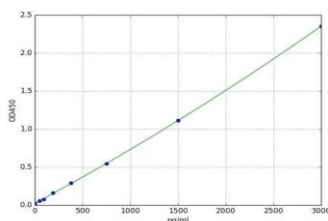
Specificity

This assay has high sensitivity and excellent specificity for detection of Ang-2. No significant cross reactivity or interference between Ang-2 and analogues was observed.

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Mean A450nm
A1, A2	Std. A (0 pg/ml)	0.133
B1, B2	Std. B (46.875 pg/ml)	0.211
C1, C2	Std. C (93.75 pg/ml)	0.284
D1, D2	Std. D (187.5 pg/ml)	0.411
E1, E2	Std. E (375 pg/ml)	0.654
F1, F2	Std. F (750 pg/ml)	0.975
G1, G2	Std. G (1500 pg/ml)	1.519
H1, H2	Std. H (3000 pg/ml)	2.123
	Sample 1	1.09

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



Calculation of results:

A dose response curve is used to ascertain the concentration of Ang-2 in unknown specimens.

- Record the A450 obtained from the printout of the microplate reader as outlined in Example 1.
- Plot the A450 for mean duplicate calibrator versus the Ang-2 concentration in pg/ml on linear graph paper. Connect the points with a best-fit curve.
- To determine the concentration of Ang-2 for an unknown, locate the average A450 of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in pg/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.09) intersects the dose response curve at 1076.36 pg/ml Ang-2 concentration (See Figure 1).

Note: If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Interpretation

- Measurements and interpretation of results must be performed by a skilled individual or trained professional.*
- Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants. The reagents for the test system procedure have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays. For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient history, and all other clinical findings.

PRINCIPLE OF THE TEST

Human Angiotensin-2 (Ang-2) ELISA kit uses sandwich-ELISA as the method. The microtiter plate provided in this kit has been pre-coated with Anti- ANG2 antibody. And the biotin conjugated Anti- ANG2 antibody was used as detection antibodies. The standards, test samples and biotin conjugated detection antibody were added to the wells and wash with wash buffer. HRP-Streptavidin was added and unbound conjugates were washed away with wash buffer. Then a TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of diluted sulphuric acid solution (stop solution) and the color change is measured spectrophotometrically at a wavelength of 450 nm. The concentration of ANG2 in the samples is then determined by comparing the OD of the samples to the standard curve.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipette (50-200 μ l) and multichannel pipet with disposable plastic tips. Reagent troughs, plate shaker (orbital shaker), plate washer (recommended) and ELISA plates Reader.

SPECIMEN COLLECTION AND STORAGE

Plasma: Collect plasma using EDTA-Na2 as an anticoagulant. Centrifuge samples for 15 minutes at 1000xg at 2 - 8°C within 30 minutes of collection. Collect the supernatant and carry out the assay immediately. Avoid hemolysis, high cholesterol samples.

Serum: Allow samples to clot for 2 hours at room temperature or overnight at 4°C before centrifugation for 20 minutes at approximately 1000xg. Collect the supernatant and carry out the assay immediately. Blood collection tubes should be disposable, non-pyrogenic, and non-endotoxin.

Notes:

- Samples should be used within 7 days when stored at 2-8°C, otherwise samples must be divided and stored at -20°C (\leq 1month) or -80°C (\leq 6months) to avoid the loss of bioactivity and contamination. Avoid repeated freeze-thaw cycles.
- Please take the samples to room temperature (18-25°C) without extra heating before performing the assay.
- Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

Assay Performance

- It is important that the time of reaction in each well is held constant to achieve reproducible results.
- Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
- Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
- If more than one (1) plate is used, it is recommended to repeat the dose response curve.
- The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
- Plate readers measure vertically. Do not touch the bottom of the wells.
- Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
- Use components from the same lot. No intermixing of reagents from different batches.
- Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed, is essential. Any deviation from ADI's IFU may yield inaccurate results.
- All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
- It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.

REAGENTS PREPARATION FOR THE ASSAY:

Wash Buffer (25X): Dilute the wash buffer with distilled water (dissolve content of 1 bottle (30 ml) into 750 ml deionized or distilled water). Some buffer components may crystallize in wash concentrate. These redissolve at room temperature. Store diluted wash buffer at 2-8°C.

Standard: 1) 3000 pg/ml of standard solution: Add 1 ml of Sample / Standard dilution buffer into one Standard tube, keep the tube at room temperature for 10 minutes and mix them thoroughly.

2) 1500pg/ml→46.875pg/ml of standard solutions: Label 6 Eppendorf tubes with 1500pg/ml, 750pg/ml, 375pg/ml, 187.5pg/ml, 93.75pg/ml, 46.875pg/ml, respectively. Aliquot 0.3 ml of the Sample /Standard dilution buffer into each tube. Add 0.3 ml of the above 3000 pg/ml standard solution into 1st tube and mix thoroughly. Transfer 0.3 ml from 1st tube to 2nd tube and mix thoroughly. Transfer 0.3 ml from 2nd tube to 3rd tube and mix thoroughly, and so on.

Note: The standard solutions are best used within 2 hours. The standard solution should be at 4°C for up to 12 hours. Or store at -20 °C for up to 48 hours. Avoid repeated freeze-thaw cycles.

Biotin- labeled Antibody Working Solution: Prepare it within 1 hour before experiment. Dilute the Biotin- labeled Antibody with Antibody Dilution Buffer at 1:100 and mix them thoroughly. (i.e. Add 1 µl of Biotin- labeled Antibody into 99 µl of Antibody Dilution Buffer)

HRP-Streptavidin Conjugate Working Solution: Prepare it within 30 minutes before experiment. Dilute the HRP-Streptavidin Conjugate with HRP-Streptavidin Conjugate dilution buffer at 1:100 and mix thoroughly. (i.e. Add 1 µl of HRP-Streptavidin Conjugate into 99 µl of HRP-Streptavidin Conjugate dilution buffer)

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 label. The whole kit stability is usually 6 months from the date of shipping under appropriate storage conditions. Do not freeze and thaw.

Quality Control:

Each laboratory should assay controls at levels in the low, normal and high range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

TEST PROCEDURE (ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE, 25-28°C, BEFORE USE). Prepare working solutions of biotin-antibody, HRP conjugate, Standard and wash buffer (see page 3). Bring all reagents and solutions to room temp. (25-28°C).

1. Organize the microplates' wells for each standard, control and test sample to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.
2. It's recommended to measure each standard and sample in duplicate. Wash plate 2 times with 350 ul wash buffer before adding standard, sample and control (blank) wells.
3. Pipette **100 µl of ANG2 standard, Blank or Sample** into the assigned well. (The blank well is added with Sample/Standard dilution buffer). Seal the plate with a cover and incubate at 37 °C for 90 min.
4. Remove the cover and discard the plate content, clap the plate on the absorbent filter papers or other absorbent material and wash plate 2 times with 350 ul wash buffer. Do NOT let the wells completely dry at any time.
5. Add **100 µl of the Biotin- labeled antibody** to all wells.
6. Mix the microplate gently for 20-30 seconds by gentle tapping against the palm. Cover and **incubate for 60 minutes** at 37°C.
7. Wash plate 3 times with 350 ul wash buffer. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.
8. Add **100 µl of HRP-Streptavidin Conjugate** to all wells. Mix the microplate gently for 20-30 seconds by tapping against the palm of your hand..
9. Cover and **incubate for 30 minutes** at 37°C.
10. Wash plate 5 times with 350 ul wash buffer. After the last wash, remove any remaining Wash Buffer by aspirating or decanting.
11. Add **90 µl of TMB substrate** reagent to all wells. Note: Always add reagents in the same order to minimize reaction time differences between wells. **DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION. Incubate at 37°C in dark within 15-30 minutes.**
12. Add **50 µl of stop solution** to each well and gently mix for 15-20 seconds. Always add reagents in the same order to minimize reaction time differences between wells.
13. **Read the absorbance in each well at 450nm. The results should be read within fifteen (15) minutes of adding the stop solution.**

Note: Dilute the samples suspected of concentration range 30000-300000 pg/ml at 1:100, 3000-30000 pg/ml at 1:10, 46.875-3000 pg/ml at 1:2 and ≤ 46.875 pg/ml not necessary to dilute, or dilute at 1:2.