

ELISA Kit Components	Amount	Cat/Part No.
Anti-Angiogenin Microwell Strip Plate	8-well strips (12)	100-161
Angiogenin Standard, lyophilized	3 vials	100-162
Anti-Angiogenin Detecting Antibody (100X)	0.15 ml	100-163
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	100-160-ANH

Human Angiogenin

**For Quantitative Determination of Angiogenin
in Solution**

INTENDED USE

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

RESEARCH USE OF THE TEST

Obesity, a common nutritional disorder, is associated with diabetes, hypertension, hyperlipidemia, cancer and many other health related problems. At least five genes, Obese (ob), diabetes (db), fat (fat), agouti yellow (Ay), and tubby (tub) have been linked to obesity. Recently, Ob genes (mouse and human) have been cloned. Obese gene encodes an adipocyte-tissue derived secreted Ob protein/Angiogenin (167 amino acid, ~16 kDa) that controls body weight homeostasis. Exogenous administration of recombinant Ob protein can reduce food intake and body weight. However, Ob protein had no effect in db/db mice suggesting a defect in Angiogenin signaling mechanism. Angiogenin exerts its effects by interacting with Angiogenin receptors (obese receptors). The Ob-R has been shown to be a product of db gene that has long been thought to encode the receptor for a weight-controlling hormone.

Purified Angiogenin has low endotoxin level and shown to be biologically effective in reducing body weight, food consumption in ob/ob mice.

ADI's Angiogenin ELISA kit is a highly sensitive sandwich type assay for the measurement of Angiogenin in serum. The assay can be adapted to measure human Angiogenin in other biological fluids such as plasma, urine, culture medium etc.

PRINCIPLE OF THE TEST

The Human Angiogenin ELISA kit is based on the binding of human angiogenin 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 Angiogenin present in the sample. Stopping Solution is added to terminate the reaction, and absorbance at 450nm is then measured using an ELISA microtiter well reader. The concentration of angiogenin in samples is calculated from a standard curve of purified recombinant human angiogenin 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 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 angiogenin immunosorbent from animals immunized with purified recombinant human angiogenin.

QUALITY CONTROL

Reagents Accurate and reproducible assay results rely on proper storage, handling and control of reagent and sample temperature. Store all reagents as indicated, and warm to room temperature only those to be used in the assay. Shelf-life of the critical reagents and samples will diminish with extended exposure to non-refrigeration, resulting in inaccurate assay results. All solutions should be clear. Cloudiness or particulates are indications of reagent contamination or instability and may interfere with proper performance of the assay. Do not use.

Sample Controls Each lab should assay internal positive control samples, which represent the lab's expected sample population and that are maintained stabilized. A Negative Diluent Control should also be run.

Standard Curve The signal generated by the standards should be continuously increasing in OD from the lowest Standard to the highest Standard, with a difference greater than 1.2 OD. Non-continuously increasing or low signals may indicate problems with technique, protocol directions and/or reagent preparation, use or stability. A Negative Diluent Control should be of lower signal than the lowest standard. Do not rely on results generated from an assay with these issues.

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 is recommended. ELISA reader and pipettes should be properly calibrated.

CALCULATION OF RESULTS

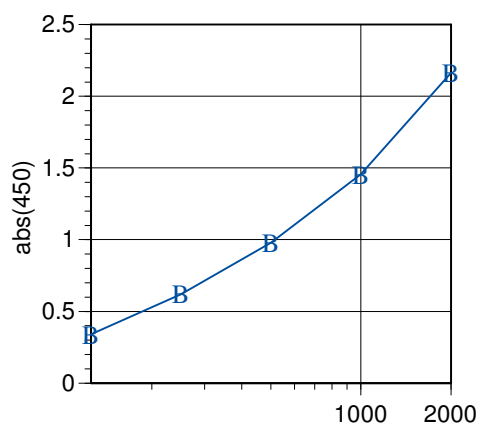
The results may be calculated using any immunoassay software package. The four-parameter curve-fit is recommended. If software is not available, Angiogenin concentrations may be determined as follows:

1. Calculate the mean OD of duplicate samples.
2. On graph paper plot the mean OD of the standards (y-axis) against the concentration (pg/ml) of Angiogenin (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 Angiogenin 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 2000 pg/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	Angiogenin pg/ml
A1, A2	Negative Diluent Control	0.023	0
B1, B2	125 pg/ml Standard	0.341	125
C1, C2	250 pg/ml Standard	0.619	250
D1, D2	500 pg/ml Standard	0.977	500
E1, E2	1000 pg/ml Standard	1.456	1000
F1, F2	2000 pg/ml Standard	2.164	2000
G1, G2	Sample [Diluted 1:10] Calculated: 10-fold dilution x 462 pg/ml = 4.62 ng/ml in sample	0.957	462



To Be Reconstituted: Store as indicated.

Component	Instructions for Use	
Human Angiogenin Standard Part No. 100-162	Three (3) vials, each containing Angiogenin lyophilized in buffer with protein, detergents and ProClin 300 as stabilizers. Keep lyophilized vials frozen until used or kit lot expires.	
Reconstitute 1 vial with 0.5ml Working Sample Diluent to provide a 2000 pg/ml Top Standard, sufficient for one entire curve. Prepare 2-fold dilutions, as follows:		
Standard	+ Diluent = Final Conc	
Reconstituted Standard	None	2000 pg/ml
250 ul of 2000pg/ml	250 ul	1000 pg/ml
250 ul of 1000pg/ml	250 ul	500 pg/ml
250 ul of 500pg/ml	250 ul	250 pg/ml
250 ul of 250pg/ml	250 ul	125 pg/ml
Use within 4 weeks of preparation (stored refrigerated).		
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.	
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.	
Anti-Human Angiogenin Detection Antibody Concentrate (100x) Part No. 100-163, 0.15ml	Biotinylated anti-human angiogenin 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 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.	

Ready For Use: Store as indicated on labels.

Component	Part No.	Amt	Contents
Anti-Human Angiogenin Microwell Strip Plate	100-161	8-well strips (12)	Coated with purified anti-human angiogenin antibodies. Return unused strips to the pouch with desiccant; re-seal and store refrigerated.
TMB Substrate	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
Stop Solution	80101	12 ml	1% sulfuric acid.

Materials Required But Not Provided:

- Pipettors and pipettes that deliver 100ul and 1-10ml. A multi-channel pipetter 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, Sample Diluent, Detection Antibody and Streptavidin-HRP 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, and 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, stored refrigerated for up to a few weeks, or frozen for long-term storage. Avoid freeze-thaw cycles.

ASSAY PROCEDURE

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

Use freshly diluted Standards as described on page 2. Dilute samples in Working Sample Diluent according to expected Angiogenin concentrations. Dilute serum and other body fluids at least 5-fold to avoid sample matrix issues; culture medium may be used neat.

ALL STEPS ARE PERFORMED 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, including 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.
 - Add 200-300ul Working Wash Solution to each well and let stand for about 5 minutes before sample addition.
 - Aspirate or dump the liquid and pat the plate dry on a paper towel.
- 2. 1st Incubation** **[100ul - 90min; 4 washes]**

 - Add 100ul of standards, samples and controls each to pre-determined wells.
 - Tap the plate gently to mix reagents and incubate for 90 minutes.
 - Wash wells 4 times and pat dry on fresh paper towels. As an alternative, an automatic plate washer is recommended. Improper washes may lead to falsely elevated signals and poor reproducibility.
- 3. 2nd Incubation** **[100ul - 60min; 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 - 30min; 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 - 15min]**

 - 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, assuring the top standard does not surpass 2 OD.
- 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.