

ELISA Kit Components	Amount	Cat/Part #
Anti-Mouse Acrp30 Microwell Strip Plate	8-well strips (12)	100-151
Mouse Acrp30 Standard, lyophilized	2 vials	100-152
Anti-Mouse Acrp30 Detecting Antibody (100X)	0.15 ml	100-153
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	100-150-ADM

Mouse Acrp30

ELISA Kit Cat. No. 100-150-ADM

For Quantitative Determination of Mouse Adiponectin (Acrp30) in Solution

QUALITY CONTROL

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 may be used. ELISA reader and pipettes should be properly calibrated.



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INTENDED USE

The Mouse Acrp30 ELISA Kit is an in vitro immunoassay for research use in the quantification of adiponectin (acrp30) in cultures of mouse cells and in appropriately qualified samples from serum, saliva, or other tissue fluids.

RESEARCH USE OF THE TEST

Adipose tissue is the largest reservoir of fuel, storing energy in the form of rapidly utilizable triglycerides. Adipocytes synthesize and store energy in periods of nutritional abundance and mobilize lipids during starvation and other times of need. In order to accomplish these complex tasks energy balance, adipocytes express many genes, including Adiponectin, involved in lipid metabolism and glucose homeostasis.

Adiponectin (Acrp30), was identified as a novel adipocyte-specific synthesized and secreted protein with structural resemblance to complement factor C1q. Like adiponectin, Acrp30 secretion is induced ~10-fold during adipocyte differentiation. Plasma levels are reduced in obese humans, and low levels are associated with insulin-resistance. Adiponectin (mouse 247 aa, rat human 244 aa; chromosome 3q27) consists of a predicted NT-signal sequence 91-14 aa), followed by a 27-aa unique region, and then by 22 perfect Gly-X-Pro or Gly-X-X collagen like repeats, and a globular segment at the C-terminus. Structurally, but at the sequence level, Adiponectin resembles other collagen-like and globular domain proteins (lung surfactant protein and hepatocytes mannan-binding proteins). Adiponectin is proteolytically cleaved at 104 aa to generate the globular Adiponectin (gAcrp30). Administration of gAcrp30 into mice fed a diet high in fat and sugar caused substantial weight loss. A marked reduction in plasma triglycerides, glucose, and free fatty acids was attributed due in part to increased fatty acid oxidation by muscle. Full length Adiponectin was less potent than gAcrp30. Therefore, gAcrp30 may open new avenues to control obesity.

PRINCIPLE OF THE TEST

The Mouse Acrp30 ELISA kit is based on the binding of mouse acrp30 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 acrp30 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 acrp30 in samples is calculated from a standard curve of purified recombinant mouse acrp30 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 mouse acrp30 immunosorbent and have been shown by ELISA to react specifically with the full-length form of acrp30 and does not detect the free C-terminal globular domain (aa111-247). The antibodies show essentially no reactivity with other mouse proteins.

Accuracy

The standards of this assay have been calibrated against highly purified, NSO-expressed, recombinant mouse acrp30.

Mouse Serum

Acrp30 Levels

Assay of stored, frozen sera of 26 individuals or pools from Swiss mice, ranged from 1.4 to 18.3 ug/ml. Normal value ranges should be established for the laboratory's expected testing populations.

Linearity of Dilution

Two (2) individual stored sera were diluted to 2 levels for testing, and concordance of the assay values were compared. The mean recovery was 95 and 98%, demonstrating linear dilution and equivalent quantification across the standard range.

Culture Medium

Mouse Acrp30 Standard (lyophilized), reconstituted and diluted with 10% Neonatal Bovine Serum, was essentially parallel with the normal Standard Curve, as shown by the 92% concordance in the table below. The standards should be diluted in the culture medium to produce linear dilution and equivalent quantification across the standard range for samples from cultured cells.

Sample	Dilution	Assay Value pg/ml	Final Value	Concordance
Mouse I	1:20k	897	18.0 ug/ml	98 %
	1:160k	116	18.6	
Mouse M	1:20k	748	15.0 ug/ml	95 %
	1:160k	104	16.7	
10% Neonatal Bovine Serum	1	1630	1630 pg/ml	92 %
	1:16	87	1392	

CALCULATION OF RESULTS

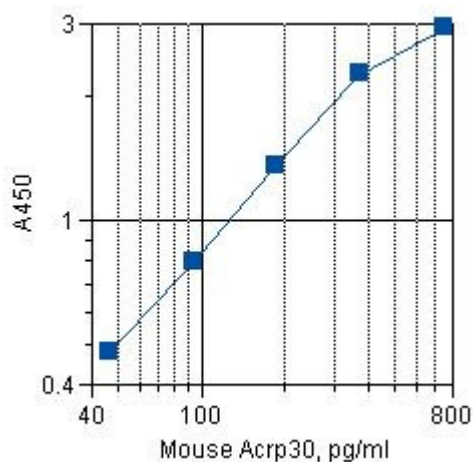
The results may be calculated using any immunoassay software package. The four-parameter curve-fit is recommended. If software is not available, acrp30 concentrations may be determined as follows:

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 acrp30 (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 acrp30 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 750 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	Acrp30 ng/ml
A1, A2	Negative Diluent Control	0.10	0
B1, B2	47 pg/ml Standard	0.48	47
C1, C2	94 pg/ml Standard	0.79	94
D1, D2	188 pg/ml Standard	1.36	188
E1, E2	375 pg/ml Standard	2.26	375
F1, F2	750 pg/ml Standard	2.95	750
G1, G2	Sample [Diluted 1:10k] Calculated: 10k-fold dilution x 171 pg/ml = 1.71 ug/ml in serum	1.23	171



To Be Reconstituted: Store as indicated.

Component	Instructions for Use	
Mouse Acrp30 Standard Part No. 100-152	Two (2) vials, each containing acrp30 lyophilized in buffer with proteins as stabilizers. Keep lyophilized vials frozen until used or kit lot expires.	
Reconstitute 1 vial with 1.0 ml Working Sample Diluent* to provide a 750 pg/ml Top Standard, sufficient for two curves. Prepare 2-fold dilutions, as follows:		
Standard	+ Diluent = Final Conc	
Reconstituted Standard	None	750 pg/ml
225 ul of 750 pg/ml	225ul	375 pg/ml
225 ul of 375 pg/ml	225ul	188 pg/ml
225 ul of 188 pg/ml	225ul	94 pg/ml
225 ul of 94 pg/ml	225ul	47 pg/ml
Use within 2 weeks of preparation. Store @ 4C.		
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-Mouse Acrp30 Detection Antibody Concentrate (100x) Part No. 100-153, 0.15ml	Biotinylated anti-mouse acrp30 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.	

* Please see **Culture Medium**, page 6.

Ready For Use: Store as indicated on labels.

Component	Part No.	Amt	Contents
Anti-Mouse Acrp30 Microwell Strip Plate	100-151	8-well strips (12)	Coated with purified anti-mouse acrp30 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 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, 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, store 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 acrp30 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 – 120 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 120 minutes.
 - Wash wells 4 times and pat dry on fresh paper towels. As an alternative, 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.