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R&D SYSTEMS

Quantikine® ELISA

Human Total Adiponectin/Acrp30 Immunoassay

Catalog Number DRP300
SRP300
PDRP300

For the quantitative determination of human total Adiponectin concentrations in cell culture supernates, serum, and plasma.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Adiponectin, alternately named Adipocyte complement-related protein of 30 kDa (Acrp30), adipoQ, adipose most abundant gene transcript 1 (apM1), and gelatin-binding protein of 28 kDa (GBP28), is an adipocyte-specific, secreted protein with potential roles in glucose and lipid homeostasis. Circulating Adiponectin levels are high, accounting for approximately 0.01% of total plasma protein (1-4). Adiponectin contains a modular structure that includes an N-terminal collagen-like domain followed by a C-terminal globular domain with significant sequence and structural resemblance to the complement factor C1q (1, 5, 6). Although they share little sequence identity, similar three-dimensional structure and certain conserved amino acid residues suggest an evolutionary link between the C1q-like domain of Adiponectin and members of the TNF superfamily (7). Adiponectin assembles into different complexes including trimers (low molecular weight), hexamers (middle molecular weight), and higher order oligomeric structures (high molecular weight) that may affect biological activity (1, 7, 8). Adiponectin is induced during adipocyte differentiation and its secretion is stimulated by insulin (1, 9). Two receptors for Adiponectin, termed AdipoR1 and AdipoR2, have been cloned (10). Although functionally distinct from G-protein-coupled receptors, the genes encode predicted proteins containing 7 transmembrane domains. AdipoR1 is highly expressed in skeletal muscle, while AdipoR2 is primarily found in hepatic tissues.

Injection of Adiponectin into non-obese diabetic mice leads to an insulin-independent decrease in glucose levels (11). This is likely due to insulin-sensitizing effects involving Adiponectin regulation of triglyceride metabolism (11). A truncated form of Adiponectin (gAdiponectin) containing only the C-terminal globular domain has been identified in the blood, and recombinant gAdiponectin has been shown to regulate weight reduction as well as free fatty acid oxidation in mouse muscle and liver (2, 12). The full-length recombinant Adiponectin protein is apparently less potent at mediating these effects (2, 12). The mechanism underlying the role of Adiponectin in lipid oxidation may involve the regulation of expression or activity of proteins associated with triglyceride metabolism including CD36, acyl CoA oxidase, AMPK, and PPARγ (12-14).

Although Adiponectin-regulation of glucose and lipid metabolism in humans is less clear, similar mechanisms may also be in place (15). A negative correlation between obesity and circulating Adiponectin has been well established (6, 16, 17), and Adiponectin levels increase concomitantly with weight loss (18). Decreased Adiponectin levels are associated with insulin resistance and hyperinsulinemia, and patients with type-2 diabetes are reported to exhibit decreased circulating Adiponectin (19, 20). Thiazolidinediones, a class of insulin-sensitizing, anti-diabetic drugs, elevate Adiponectin in insulin-resistant patients (21). In addition, high Adiponectin levels are associated with a reduced risk of type-2 diabetes (22). Using magnetic resonance spectroscopy it has been demonstrated that intracellular lipid content in human muscle negatively correlates with Adiponectin levels, potentially due to Adiponectin-induced fatty acid oxidation (15).

Adiponectin may also play anti-atherogenic and anti-inflammatory roles. Adiponectin plasma levels are decreased in patients with coronary artery disease (20). Furthermore, neointimal thickening of damaged arteries is exacerbated in Adiponectin-deficient mice and is inhibited by exogenous Adiponectin (23). Adiponectin inhibits endothelial cell expression of adhesion molecules *in vitro*, suppressing the attachment of monocytes (24). In addition, Adiponectin negatively regulates myelomonocytic progenitor cell growth and TNF-α production in macrophages (25, 26).

The Quantikine® Human Total Adiponectin/Acrp30 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure total (low, middle, and high molecular weight) human Adiponectin in cell culture supernates, serum, and plasma. It contains NS0-expressed recombinant human Adiponectin and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human Adiponectin showed linear curves that were parallel to the standard curves obtained using the Quantikine® kit standards. These results indicate that this kit can be used to determine relative mass values for natural human Adiponectin.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for the human Adiponectin globular domain has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Adiponectin present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for the human Adiponectin globular domain is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of Adiponectin bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- It is important that the calibrator diluent selected for the standard curve be consistent with the samples being assayed.
- If samples generate values higher than the highest standard, dilute the samples with the appropriate calibrator diluent and repeat the assay.
- Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine® Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	CATALOG # DRP300	CATALOG # SRP300	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human Adiponectin Microplate	892517	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human Adiponectin globular domain.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Human Adiponectin Standard	892519	2 vials	12 vials	Recombinant human Adiponectin in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Use a fresh standard for each assay. Discard after use.
Human Adiponectin Conjugate	892518	1 vial	6 vials	21 mL/vial of a monoclonal antibody specific for human Adiponectin globular domain conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1W	895117	1 vial	6 vials	11 mL/vial of a buffered protein base with preservatives.	
Calibrator Diluent RD5-5	895485	1 vial	6 vials	21 mL/vial of a buffered protein base with preservatives. *For cell culture supernate samples.*	
Calibrator Diluent RD6-39	895824	3 vials	18 vials	21 mL/vial of a buffered protein base with preservatives. *For serum/plasma samples.*	
Wash Buffer Concentrate	895003	1 vial	6 vials	21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservatives. *May turn yellow over time.*	
Color Reagent A	895000	1 vial	6 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	6 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	1 vial	6 vials	6 mL/vial of 2 N sulfuric acid.	
Plate Sealers	N/A	4 strips	24 strips	Adhesive strips.	

* Provided this is within the expiration date of the kit.

DRP300 contains sufficient materials to run an ELISA on one 96 well plate.
SRP300 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PDRP300). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Refer to the PharmPak Contents section for specific vial counts.

PHARMPAK CONTENTS

Each PharmPak contains reagents sufficient for the assay of 50 microplates (96 wells/plate). The package inserts supplied are the same as those supplied in the single kit packs and because of this, a few minor differences related to the number of reagents and their container sizes should be noted.

- Sufficient material is supplied to perform at least 50 standard curves; reuse of each vial may be required. The number of vials, and the number of standard curves obtained per vial will vary with the analyte.
- Wash Buffer 25X Concentrate is bulk packed in 125 mL bottles containing 100 mL, and not in the glass vials described in the package insert. **Note:** *Additional wash buffer is available for purchase (R&D Systems®, Catalog # WA126).*

The reagents provided in this PharmPak are detailed below.

PART	PART #	QUANTITY
Human Adiponectin Microplate	892517	50 plates
Human Adiponectin Conjugate	892518	50 vials
Human Adiponectin Standard	892519	25 vials
Calibrator Diluent RD5-5	895485	50 vials
or		
Calibrator Diluent RD6-39	895824	150 vials
Assay Diluent RD1W	895117	50 vials
Color Reagent A	895000	50 vials
Color Reagent B	895001	50 vials
Wash Buffer Concentrate, 25X	895126	9 bottles
Stop Solution	895032	50 vials
Plate Sealers	N/A	100 sheets
Product Insert	751018	2 booklets

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 500 mL graduated cylinder.
- Test tubes for dilution of standards and samples.
- Human Total Adiponectin Controls (optional; R&D Systems®, Catalog # QC237).

PRECAUTIONS

Calibrator Diluent RD6-39 contains sodium azide which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

The Stop Solution provided with this kit is an acid solution.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

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 at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*

SAMPLE PREPARATION

Serum and plasma samples require a 100-fold dilution. A suggested 100-fold dilution is 10 µL of sample + 990 µL of Calbrator Diluent RD6-39.

REAGENT PREPARATION

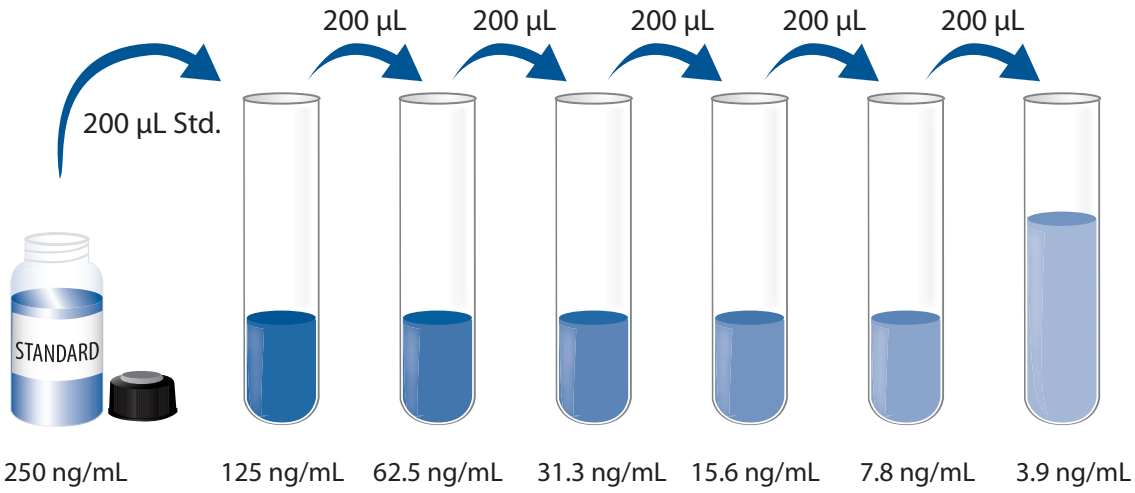
Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Human Adiponectin Standard - Refer to the vial label for reconstitution volume. Reconstitute the Human Adiponectin Standard with Calibrator Diluent RD5-5 (*for cell culture supernate samples*) or Calibrator Diluent RD6-39 (*for serum/plasma samples*). This reconstitution produces a stock solution of 250 ng/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 200 µL of Calibrator Diluent RD5-5 (*for cell culture supernate samples*) or Calibrator Diluent RD6-39 (*for serum/plasma samples*) into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Human Adiponectin Standard (250 ng/mL) serves as the high standard. The appropriate calibrator diluent serves as the zero standard (0 ng/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of Assay Diluent RD1W to each well.
4. Add 50 µL of standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature. A plate layout is provided to record standards and samples assayed.
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 (400 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 200 µL of Human Adiponectin Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well.
   For Cell Culture Supernate samples: Incubate for **20 minutes** at room temperature.
   For Serum/Plasma samples: Incubate for **30 minutes** at room temperature.
   Protect from light.
9. Add 50 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.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Samples may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

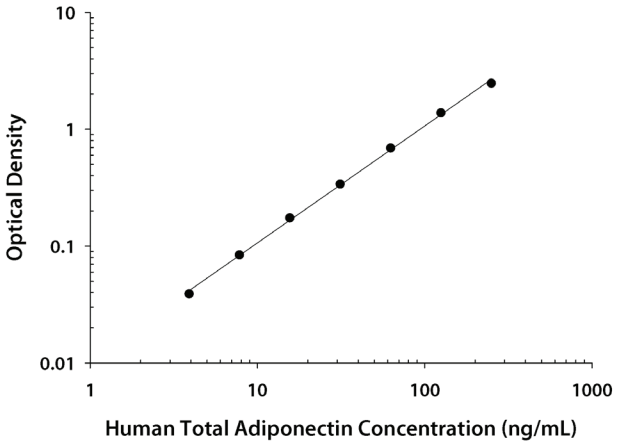
Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the human Adiponectin concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

If the samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

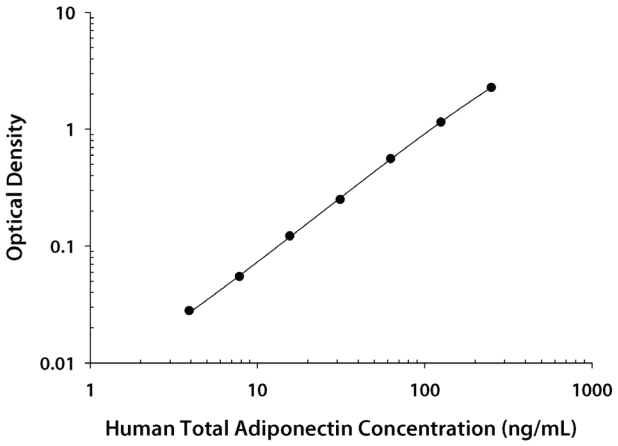
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(ng/mL)	O.D.	Average	Corrected
0	0.011 0.012	0.012	—
3.9	0.051 0.051	0.051	0.039
7.8	0.092 0.100	0.096	0.084
15.6	0.185 0.187	0.186	0.174
31.3	0.339 0.362	0.351	0.339
62.5	0.692 0.711	0.702	0.690
125	1.382 1.401	1.392	1.380
250	2.464 2.496	2.480	2.468

SERUM/PLASMA ASSAY



(ng/mL)	O.D.	Average	Corrected
0	0.013 0.014	0.014	—
3.9	0.042 0.042	0.042	0.028
7.8	0.068 0.070	0.069	0.055
15.6	0.132 0.140	0.136	0.122
31.3	0.248 0.282	0.265	0.251
62.5	0.572 0.573	0.573	0.559
125	1.155 1.175	1.165	1.151
250	2.216 2.356	2.286	2.272

PRECISION

Intra-Assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision between assays)
Three samples of known concentration were tested in forty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

CELL CULTURE SUPERNATE ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (ng/mL)	12.5	45.3	91.5	12.7	47.1	100
Standard deviation	0.41	1.27	4.56	1.00	2.80	6.70
CV (%)	3.3	2.8	5.0	7.9	5.9	6.7

SERUM/PLASMA ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (ng/mL)	19.8	69.9	143	20.5	74.4	157
Standard deviation	0.50	2.40	6.76	1.40	4.30	10.8
CV (%)	2.5	3.4	4.7	6.8	5.8	6.9

RECOVERY

The recovery of human Adiponectin spiked to levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	101	88-112%

SENSITIVITY

Eighty assays were evaluated and the minimum detectable dose (MDD) of human Adiponectin ranged from 0.079-0.891 ng/mL. The mean MDD was 0.246 ng/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human Adiponectin were serially diluted with the appropriate calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=4)	Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)
1:2	Average % of Expected	104	99	95	99
	Range (%)	101-107	92-103	86-102	93-106
1:4	Average % of Expected	103	98	96	99
	Range (%)	101-106	91-101	89-104	92-109
1:8	Average % of Expected	103	102	97	104
	Range (%)	101-104	98-105	84-102	97-107
1:16	Average % of Expected	103	105	101	104
	Range (%)	100-104	96-115	85-107	103-105

*Samples were diluted prior to assay as directed in the Sample Preparation section.

CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant human Adiponectin produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human Adiponectin in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=60)	6641	865 - 21,424	3665
EDTA plasma (n=35)	5548	1198 - 19,973	3557
Heparin plasma (n=35)	6026	1359 - 20,691	3728

Cell Culture Supernates - Human peripheral blood leukocytes (1 x 10^6 cells/mL) were cultured in DMEM supplemented with 5% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 µg/mL PHA. Aliquots of the cell culture supernate were removed and assayed for levels of total human Adiponectin. No detectable levels were observed.

SPECIFICITY

This assay recognizes natural and recombinant (low, middle, and high molecular weight) human total Adiponectin.

The factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range recombinant human Adiponectin control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

4-1BB
APRIL
BAFF/BLyS
CD27
CD30 Ligand
CD40 Ligand
Fas Ligand
GITR Ligand
LIGHT
LT-α1/β2
LT-α2/β1
OX40 Ligand
TNF-α
TNF-β
TRAIL
TRANCE
TWEAK
VEGI

Recombinant mouse:

Adiponectin
CD27 Ligand
CD30 Ligand
Fas Ligand
LT-α1/β2
LT-α2/β1
OX40 Ligand
TNF-α
TNF-α (truncated)
TRANCE

Recombinant porcine:

TNF-α

Recombinant rat:

TNF-α

Natural proteins:

human Complement C1q

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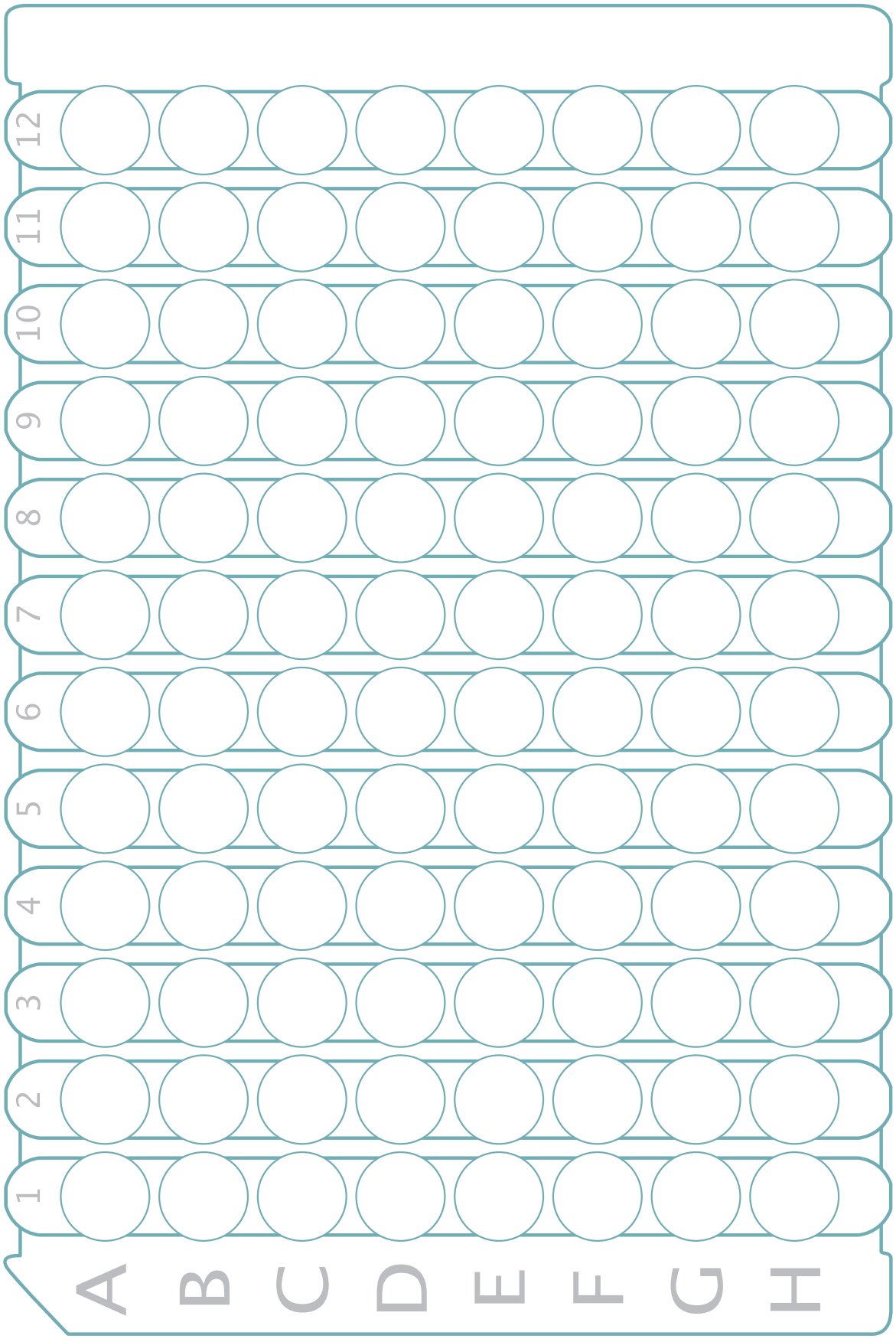
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



NOTES