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)

100-300-SCR Serum Creatinine ELISA kit (colorimetric, all species), 96 tests, quantitative

100-305-SCR Serum Creatinine ELISA kit (colorimetric, all species), 2x96 tests, quantitative

100-310-ADM Human Asymmetrical Dimethylarginine (ADMA) ELISA Kit, 96 tests

100-320-CIT Human Citrulline (CIT) ELISA Kit, 96 tests

100-330-ARG Human Arginine (Arg) ELISA Kit, 96 tests

Instruction Manual No. M-100-330-ARG

Arginine (ARG)

ELISA KIT #100-330-ARG

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

For In Vitro Research Use Only

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

Arginine ELISA KIT Cat. No. 100-330-ARG

For Quantitative Determination of arginine in serum, plasma

Kit Contents: (reagents for 96 tests)

Components	
coated microwell strip plate (96 wells);#100331	1 plate
ARG **Standard**, Lyophilized; 0.5 ml, #100332A	2 vials
Standard Diluent, 20 ml, #100333	1 bottle
Detection **Reagent A**, 120 **µl** #100334A	1 vial
Detection **Reagent B**, 120 **µl** #100334B	1 vial
Assay **Diluent A**, 12 ml # 100335A	1 bottle
Assay **Diluent B**, 12 ml # 100335B	1 bottle
TMB Substrate, 9 ml, #100330TM	1 bottle
Wash buffer (30X), 20 ml, dilute 1:30 with distilled water #100330WB	1 bottle
Stop solution, 6 ml, #100330-ST	1 bottle
Plate sealer/cover for ELISA plates	4
Complete Instruction Manual, M-100-330-ARG	1

The Arginine ELISA kit is a competitive inhibition enzyme immunoassay for the measurement of arginine in serum, plasma, tissue homogenates and other biological fluids. For in vitro research use only.

Introduction

L-arginine is an amino acid that is obtained from the diet and is necessary for the body to make proteins. L-arginine is used for heart and blood vessel conditions including congestive heart failure (CHF), chest pain, high blood pressure, and coronary artery disease. Arginine is the immediate precursor of nitric oxide (NO), urea, ornithine, and agmatine; is necessary for the synthesis of creatine; and can also be used for the synthesis of polyamines (mainly through ornithine and to a lesser degree through agmatine, citrulline, and glutamate. As a precursor of nitric oxide, arginine may have a role in the treatment of some conditions where vasodilation is required. Nitric oxide causes blood vessels to open wider for improved blood flow. L-arginine also stimulates the release of growth hormone, insulin, and other substances in the body.

Arginase is an enzyme that converts L-arginine to urea and L-ornithine. The two isoforms of arginase are expressed in a number of tissues including the lung and are thought to reduce NO production from NOS by limiting the availability of substrate L-arginine. Thus arginase may represent a target for interventions aiming to increase L-arginine availability for NOS and NO production. Inhibition of arginase in animal models of allergic airway inflammation, for instance, resulted in anti-inflammatory effects and abrogation of airway remodeling and hyper responsiveness to methacholine in these animals, presumably by increasing L-arginine availability for NOS and increased NO formation.

Arginine measurement, therefore, can provide important physiological or disease information.

PERFORMANCE CHARACTERISTICS

Detection range : 1.23-100µg/mL. .

Sensitivity:

The minimum detectable dose of arginine is typically ~0.45 µg/mL. The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest protein concentration that could be differentiated from zero. It was determined by subtracting two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

SPECIFICITY:

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

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

Matrix	Recovery range (%)	Average(%)
serum(n=5)	85-97	92
EDTA plasma(n=5)	94-103	98
heparin plasma(n=5)	82-96	87

LINEARITY:

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

Sample dilution	1:2	1:4	1:8	1:16
serum(n=5)	83-99%	89-104%	79-97%	80-93%
EDTA plasma(n=5)	86-95%	85-96%	88-102%	95-105%
heparin plasma(n=5)	97-105%	82-91%	87-101%	90-103%

PRECISION:

Intra-assay:(Precision within an assay): 3 samples with low, middle and high level arginine were tested 20 times on one plate, respectively.

Inter-assay:(Precision between assays): 3 samples with low, middle and high level arginine were tested on 3 different plates, 8 replicates in each plate.

CV(%) = SD/meanX100

Intra-Assay:: CV<10%

Inter-Assay: CV<12%

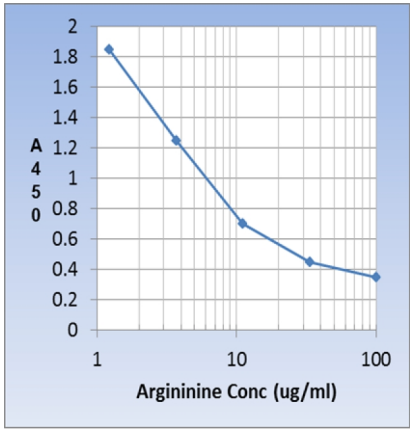
Species Reactivity

Arginine is a naturally occurring amino acids and it is found in all species. The ELISA kit has been tested in human samples but the kit should work in mouse, rat, and other species as arginine antibodies are not species specific.

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Mean A_{450nm}	
A1, A2	**Blanks** (0 ug/ml)	1.95	
B1, B2	**Std. E** (1.23 ug/ml)	1.85	
C1, C2	**Std. D** (3.70 ug/ml)	1.25	
D1, D2	**Std. C** (11.11 ug/ml)	0.700	
E1, E2	**Std. B** (33.33 ug/ml)	0.45	
F1, F2	Std A (100 ug/ml)	0.35	

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.



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A typical std. assay curve (do not use this for calculating sample values). Actual curve may vary slightly or day to day curves.

CALCULATION OF RESULTS

Determine average of the duplicate A450 readings for each standard, control, and samples. Create a standard curve on log-log or semi-log graph paper, with the log of arginine concentration on the y-axis and absorbance on the x-axis. Use point to point curve (do not force a straignt line) or 4-point-curve. Calculate the sample values from the standard curve. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

PRINCIPLE OF THE TEST

This assay employs the competitive ELISA technique. A monoclonal antibody specific to arginine has been pre-coated onto a microplate. A competitive inhibition reaction is launched between biotin labeled arginine and unlabeled arginine (Standards or samples) with the pre-coated antibody specific to arginine. After incubation the unbound conjugate is washed off. Next, avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. The amount of bound HRP conjugate is reverse proportional to the concentration of arginine in the sample. After addition of the substrate solution, the intensity of color developed is reverse proportional to the concentration of arginine in the sample.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (25-100 µl) and multichannel pipet with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site. TMB (substrate), H2SO4 (stop solution), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates). All waste material should be properly disinfected before disposal. Avoid contact with the stop solution (1N sulfuric acid).
Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site: TMB (substrate), H2SO4 (stop solution), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).
http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10

SAMPLE COLLECTION AND STORAGE:

Serum - Use a serum separator tube and allow samples to clot for 2 hours at room temperature or overnight at 4oC before centrifugation for 20 minutes at ~1000×g. Assay freshly prepared serum immediately or store samples in aliquot at -20oC or -80oC for later use. Avoid repeated freeze/thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2 - 8oC within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20oC or -80oC for later use. Avoid repeated freeze/thaw cycles.

Tissue homogenates - The preparation of tissue homogenates will vary depending upon tissue type. For this assay, tissues were rinsed in ice-cold PBS (0.01mol/L,pH 7.0-7.2) to remove excess blood thoroughly and weighed before homogenization. Minced the tissues to small pieces and homogenized them in 5-10mL of PBS with a glass homogenizer on ice (Micro Tissue Grinders woks, too). The resulting suspension was sonicated with an ultrasonic cell disrupter or subjected to two freeze-thaw cycles to further break the cell membranes. After that, the homogenates were centrifugated for 5 minutes at 5000×g. Remove the supernate and assay immediately or aliquot and store at ≤-20oC.

Other biological fluids - Centrifuge samples for 20 minutes at 1000×g. Remove particulates and assay immediately or store samples in aliquot at -20oC or -80oC. Avoid repeated freeze/thaw cycles.

Note: 1. Samples to be used within 5 days may be stored at 4oC, otherwise samples must be stored at -20oC (≤1 month) or -80oC (≤2 months) to avoid loss of bioactivity and contamination.
2. Sample hemolysis will influence the result, so hemolytic specimen should not be detected.
3. When performing the assay, bring samples to room temperature.

REAGENT PREPRATION:

1. **Standard Preparation**

Arginine standard is supplied as stock lyophilized powder. Reconstitute the **stock Standard** with 0.5 mL of **Standard Diluent,** kept for 10 minutes at room temperature, shake gently (not to foam). The concentration of the standard A in the stock solution is **100µg/mL**. Prepare additional standards by 3-fold serial dilution as follow:

Stds	Volume	Std Diluent	Total volume	Final. Concn
Std A	500 ul	0	500 ul	100 ug/ml
Std B	100 ul of A	200 ul	300 ul	33.00 ug/ml
Std C	100 ul of B	200 ul	300 ul	11.11 ug/ml
Std D	100 ul of C	200 ul	300 ul	3.70 ug/ml
Std E	100 ul of D	200 ul	300 ul	1.23 ug/ml
Std F (blanks)		200 ul	300 ul	0

Mix each tube thoroughly before the next transfer. In the above example, only 200 ul of the stds (B-E) will remain that will be sufficient to run 1 test in duplicate. Store reconstituted and diluted standards at 4oC. Do not use the working stds (B-E) beyond the assay date and prepare fresh stds if necessary.

3. 1X **Detection Reagent A:** Briefly spin or centrifuge the stock Detection A before use. Dilute 1:100 with **Assay Diluent A (**e.g. 50 ul of stock A in 4950 ul of Diluent A). You will need 50 ul/well or a total of 5 ml for full plate assay. Store at 4oC until used.

2. 1X **Detection Reagent B:** Briefly spin or centrifuge the stock Detection B before use. Dilute 1:100 with **Assay Diluent B (**e.g. 50 ul of stock A in 4950 ul of Diluent B). You will need 50 ul/well or a total of 5 ml for full plate assay. Store at 4oC until used.

4. **Wash Solution** - Dilute 20 mL of stock (30X) with 580mL of deionized or distilled water to prepare 600mL of Wash Solution (1X). Store at 4oC until used.

SAMPLE PREPARATION:

1. The user should calculate the possible amount of the samples used in the whole test.

2. Unknown samples values vary widely so it is a good idea to run just a few samples to get general idea. Sampels with out of range values should be diluted by 0.01mol/L PBS (PH=7.0-7.2) and retested.

3. Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts from certain chemicals.

4. Due to the possibility of mismatching between antigen from other origin and antibody used in our kits (e.g.,antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by our products.

5. Influenced by the factors including cell viability, cell number or sampling time, samples from cell culture supernatant may not be detected by the kit.

6. Fresh samples without long time storage is recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

STORAGE AND STABILITY

1. **For unopened kit:** All the reagents should be kept according to the labels on vials. The **Standard**, **Detection Reagent A, Detection Reagent B** and the **96-well strip plate** should be stored at -20oC upon receipt while the others should be at 4 oC.
1. **For opened kit:** When the kit is opened, the remaining reagents still need to be stored according to theabove storage condition. Besides, please return the unused wells to the foil pouch containing the desiccant pack, and reseal along entire edge of zip-seal.
2. **Note:** It is highly recommended to use the remaining reagents within 1 month provided this is within the expiration date of the kit. For the expiration date of the kit, please refer to the label on the kit box. All components are stable until this expiration date.

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).* **Prepare standards, Detection reagents A/B, and Dilute wash buffer.** Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag.

Important: If you have not used this kit before, we recommend to use 1 or 2 strips to run the standards alone to get familiar with the test and not runt the risk of making mistakes and lose sample or the whole kit.

Step 1. Pipet **50 µl** of **standard** and **samples in duplicate** into appropriate well & then add **50 µl 1X Detection reagent A**. Mix gently, cover the plate and Incubate for **60 min at 37oC**. (Detection reagent may appear cloudy).

Step 2 **Aspirate & wash** 3 times with 1 X wash buffer (350 ul/wash). After the last wash, invert the plate and tap over the fresh paper towels to remove any residual wash solution.

Step 3 Add **100 µl** of **1x detection reagent B,** Mix gently, cover the plate and Incubate for **30 min at 37oC**..

Step 4 Aspirate & wash 5 times with 1 X wash buffer as in step 2.

Step 5 Add **90 µl** substrate solution & Incubate for 15 min. Blue color develops in standards and samples. Note: It is possible to vary the reaction time ± 5 mins so as to get the maximum color A450 of 2.00-2.50 or within the linear range.

Step 6. Pipet **50 ul of stop solution** into each well and mix gently (blue color turns yellow). **Measure at 450 nm**. Determine Arginine concentration in each sample using the standards (results are expressed in µg/ml). Note: Maximum color will be obtained in the blanks and the lowest stds and the least in the highest standards as the color in inversely proportion to Arginine concentration.

Notes and Recommendations:

1. Making serial dilution in the wells directly is not recommended.
2. Prepare standard within 15 minutes before assay. Please do not dissolve the reagents at 37oC directly.
3. Detection Reagent A and B are sticky solutions, therefore, slowly pipette them to reduce the volume errors.
4. Please carefully reconstitute Standards or working Detection Reagent A and B according to the instruction, and avoid foaming and mix gently until the crystals are completely dissolved. To minimize imprecision caused by pipetting, use small volumes and ensure that pipettors are calibrated. It is recommended to suck more than 10µL for once pipetting.
5. The reconstituted Standards, Detection Reagent A and Detection Reagent B can be **used only once**.
6. If crystals have formed in the Wash Solution concentrate (30×), warm to room temperature and mix gently until the crystals are completely dissolved.
7. Contaminated water or container for reagent preparation will influence the detection result.