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-100-320-CIT

Citrulline (CIT) ELISA KIT

Cat # 100-320-CIT, 96 Tests

For Quantitative Determination of citrulline in human serum, blood plasma, tissue homogenate, feces, urine and body fluid (also applicable in animal samples (mouse, rat etc).

For In Vitro Research Use Only

ALPHA DIAGNOSTIC INTERNATIONAL

6203 Woodlake Center Drive • San Antonio• Texas 78244 • USA.
Phone (210) 561-9515 • Fax (210) 561-9544
Toll Free (800) 786-5777
Email: service@4adi.com
Web Site: www.4adi.com

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

Human Citrulline ELISA KIT Cat. No. 100-320-CIT

Kit Contents: (reagents for 96 tests)

Components	
Human Citrulline coated microwell strip plate (96 wells);#100321	1 plate
Citrulline **Std. A**, 0.5 ml, 128 nmol/ml, #100322A (perform 2-fold serial dilution to make 64, 32, 16, 8, and 4 nmol/ml stds).	1 vial
Standard Diluent 3 ml, #100323	1 vial
Streptavidin-HRP, 6 ml, #100324	1 vial
Anti-Citrulline-biotin IgG, 1 ml #100325	1 vial
HRP substrate Solution A, 6 ml # 100320SA	1 bottle
HRP substrate Solution B, 6 ml # 100320SB	1 bottle
Wash buffer (30X), 20 ml, dilute 1:30 with distilled water #WB-30	1 bottle
Stop solution, 6 ml, #ST-10	1 bottle
ELISA Plate covers	2
Complete Instruction Manual, M-100-320-CIT	1

Intended use:

Citrulline ELISA kit is for Quantitative Determination of citrulline in human serum, blood plasma and other related biological liquid. It can be adapted for other species as well (mouse, rat etc). For In Vitro Research Use Only (RUO).

Introduction

L-citrulline is a naturally occurring amino acid. It is found in some foods like watermelons and is also produced naturally by the body. Citrulline is a key intermediate in the urea cycle, the pathway by which mammals excrete ammonia. Nitric oxide (NO) is an intra- and intercellular signaling molecule. It reacts with free radicals, metalloproteins and specific amino acid residues of proteins. NO plays an important role in the regulation of vascular tone. Endothelial NO (eNO) is produced by the vascular endothelium. It diffuses to neighboring vascular smooth muscle cells (VSMC), where NO activates soluble guanylate cyclase (sGC), which subsequently increases the intracellular cGMP production from GTP, and which in turn causes relaxation of smooth muscle and vasodilatation. Thus, functional changes of the endothelium in coronary artery disease may be an important factor in the development of vasospasm, ischemia and thrombosis. L-citrulline acts as a surrogate marker for NO. NO is synthesized in the citrulline-NO-cycle when L-arginine is oxidized to citrulline by NO synthase (NOS). In the second part of the urea cycle, arginine is re-synthesized from citrulline. The NOS catalyzed formation of L-citrulline and NO proceeds in two steps, whereby the product stochiometry of L-citrulline and NO is 1:1. Thus, the conversion of L-arginine to L-citrulline can be used as a surrogate marker for the NO synthesis. Pathologic high levels of citrulline serve as an indicator of nitrosative stress.

PERFORMANCE CHARACTERISTICS

Detection Range: The detection range of this kit is 0.5-100 nmol/ml.

Sensitivity: The sensitivity of this kit is less than 0.23 nmo/ml.

Specificity: This kit recognizes recombinant and natural human CIT, no significant cross-reactivity or interference was observed.

Reproducibility: Intra-assay CV (%) and Inter-assay CV (%) are less than 15%.

Species Reactivity

Citrulline 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 citrulline and citrulline antibodies are not species specific.

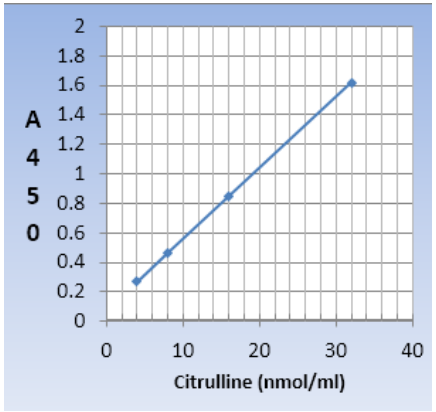
QUALITY CONTROL

Each laboratory should utilize controls at several levels to monitor assay performance. The controls should be treated as unknown. Values obtained should be in a agreement with the assigned values of the control. Controls can be obtained from commercially available sources but should not contain sodium azide as preservative.

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples nmol/ml	Mean A_{450nm}	Net A450nm
A1, A2	**Std. A** (0)	0.098	-
B1, B2	**Std. B** (4)	0.276	0.176
C1, C2	**Std. C** (8)	0.368	0.268
D1, D2	**Std. D** (16)	0.565	0.464
E1, E2	**Std. E** (32)	0.952	0.852
F1, F2	**Std. F** (64)	1.719	1.619

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.



MA/8_ADI_ELISA_Kits/100-320-CIT-Citrulline-ELISA-Graph

A typical std. assay curve (do not use this for calculating sample values). Actual A450 values may differ slightly from the above curve and from day to day. Use the standard plot generate from each test.

CALCULATION OF RESULTS

Calculate the Net A450 values of the duplicate (deduct blanks/zero values). Plot the Citrulline standard concentration versus the net A450 using a 4-point curve or linear curves. Calculate the samples values from the standard curve.

PRINCIPLE OF THE TEST

Citrulline ELISA kit is based on binding of citrulline from samples to antibody coated on the microwell plate and to the enzyme labeled antibody. Higher concentrations of citrulline in the samples result in increased binding of enzyme (HRP) labeled antibody to the microwell plate. After a washing step, chromogenic substrate is added and color developed. The enzymatic reaction (color) is proportional to the amount of citrulline present in the sample. Adding stopping solution terminates the reaction. Absorbance is then measured on a microtiter well ELISA reader at 450 nm. and the concentration of citrulline in samples and control is read off the standard curve.

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

SAMPLES COLLECTION AND STORAGE:

Samples contain azide cannot be used as it will inhibit the enzyme.

Serum - Allow samples to clot for two hours at room temperature or overnight at 2 - 8°C before centrifugation for 20 minutes at approximately 1000 × g (or 3000 rpm). Assay immediately or store samples in aliquot at -20o-80°C. Avoid repeated freeze/thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000 × g (or 3000 rpm) at 2 - 8°C within 30 minutes of collection. Assay immediately or store samples in aliquot at -20°C or -80°C. 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 (0.02mol/L, pH 7.2-7.4). Remove excess blood thoroughly and weighed before homogenization. Minced the tissues to small pieces and homogenized them in a certain amount of PBS (Usually 10mg tissue to 100µl PBS.) with a glass homogenizer on ice. The resulting suspension was subjected to ultrasonication or to two freeze-thaw cycles to further break the cell membranes. After that, centrifugate homogenates for 15 minutes at 1500×g (or 5000 rpm). Assay immediately or store samples at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

Feces - Collect by sterile tube. Dilute the samples in a certain amount of PBS (0.02mol/L, pH 7.2-7.4). Usually 10mg feces to 100µl PBS. Fully shaking and after 10 minutes' standing, Centrifuge samples for approximately 20 minutes at 1000 × g (or 3000 rpm). Collect the supernatants carefully, assay immediately or store samples at -20°C or -80°C.

Urine and Body Fluids - Collect by sterile tube. 1000 × g (or 3000 rpm) for approximately 20 minutes. Collect the supernatants carefully, assay immediately or store samples at -20°C or -80°C. When sediments occurred during storage, centrifugation should be performed again. When collecting **Pleuroperitoneal Fluid** and **Cerebrospinal Fluid**, please follow the procedures above-mentioned.

Note: 1. **Samples** to be used within 5 days may be stored at 4°C, otherwise samples must be stored at -20°C (≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination.

2. Sample hemolysis will influence the result, so the samples should be centrifuged adequately and no hemolysis or granule was allowed.

3. When performing the assay, bring samples to room temperature.

REAGENT PREPRATION

Dilute wash buffer (1:30) with distilled water (20 ml stock in total of 600 ml). Store at 4oC.

Standard Dilution instructions:

The standard is provided as 1 vial of 128 nmol/ml. It should be diluted with 2-fold serial dilution in standard diluent only. Prepare 120 ul of each standards and use 50 ul x 2 (duplicate test). Dilute the standard as needed and keep the 128 nmol/ml stock undiluted.

	Intimal Concentration	Standard diluent	Final Concentration
Std 1	120 ul of 128 nmol/ml	120 ul	64 nmol/ml
Std 2	120 ul of 64 nmol/ml	120 ul	32 nmol/ml
Std 3	120 ul of 32 nmol/ml	120 ul	16 nmol/ml
Std 4	120 ul of 16 nmol/ml	120 ul	8 nmol/ml
Std 5	120 ul of 8 nmol/ml	120 ul	4 nmol/ml

Run standards 1-5 in the test.

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8^{O}C until the expiration date printed on the label.

NOTES: Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. Plate readers measure absorbance vertically. do not touch the bottom of the wells.

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 run the risk of making mistakes and lose sample or the whole kit.

	Blanks	Standards	Samples
Std or sample	50 ul	50 ul	40 ul
Anti-Citrulline antibodies	0	0	40 ul
Streptavidin-HRP	0	50	50
Mix gently, cover the plate and incubate for **60 min** at **37oC** on an orbital shaker			
wash the wells 4 times			
Mix **Substrate A and B** (1:1 vol/vol)	100 ul	100 ul	100 ul
incubate for **10-15 min at 37oC** on a shaker (blue color in stds/samples)			
50 ul of stop solution to all wells (blue turns yellow)			
Read the plate at 450nm or use a refs filter of 630nm if available.			

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).* **Dilute wash buffer. Prepare stds 1-5 for the test (see page 3).** Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag.

Step 1. **Blanks: k**eep two wells as blanks and add **50 ul** sample diluent only**.**

Standards: Pipet **50µl** of **standard** in duplicate into respective wells. Add **50 ul Streptavidin-HRP** per well.

For samples: Add **40 µl** sample and then **10 µl Cit-antibodies** all sample wells. Add **50 ul Streptavidin-HRP** to all sample wells. Add Mix gently, cover the plate and incubate for **60 min** at **37oC** on an orbital shaker.

Step 2. **Aspirate or remove well contents** and wash the wells 4 times using 300-350 ul/wash buffer/well. After the last wash, invert the plate over fresh paper towels and tap a few times to remove traces of wash buffer.

Step 3. Add **50 ul substrate solution A followed by 50 ul solution B**, mix gently for a few seconds by tapping the plate against the palm. Alternatively, mix 1:1 solution of A+B in required volume, mix and dispense 100 ul/well. Cover the plate, and incubate for **10 min at 37oC** (blue color develops in standards and samples). **Notes**: It is possible to change the incubation time +5 mins so as to get the maximum yellow color A450nm to about 1.5-2.5 or within the linear range of the ELISA reader.

Step 4. Pipet **50 ul of stop solution** into each well and mix gently (blue color turns yellow). **Measure at 450 nm**. Determine Citrulline concentration in each sample using the standards (results are expressed in nmol/ml).