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)

#1850	Human Cortisol	**#1860**	Human Progesterone
#1865	Human Pregnolone	**#1875**	Human Aldosterone
#1880	Human Testosterone	**#1885**	Human free Testosterone
#1910	Human Androstenedione	**#1920**	Human Estradiol
#1925	Human Estrone	**#1940**	Dihydrotestosterone (DHT)
#1950	Human DHEA-sulphate (DHEA-S)		
#3400	Human serum Neopterin		

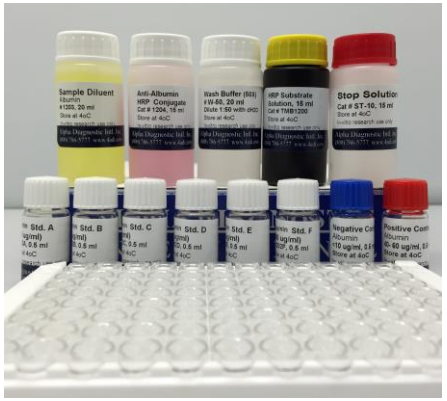
#3000	Human Rheumatoid Factors IgM (RF)
#3100	Human anti-dsDNA
#3200	Anti-Nuclear Antibodies (ANA)

Instruction Manual No. M-1200

Human Albumin

ELISA KIT Cat. No. 1200, 96 Tests

For Quantitative Determination of Human Albumin in Urine



For In Vitro Research Use Only

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Human Albumin ELISA KIT Cat. No. 1200

For Quantitative Determination of Human Albumin In Urine

Kit Contents: (reagents for 96 tests)

Components	
Human Albumin coated microwell strip plate (96 wells);#1201	96 wells (1 plate)
Albumin **Std. A**, 0.5 ml, 0.15 ug/ml, #1202A	1 vial
Albumin **Std. B**, 0.5 ml, 1.5 ug/ml, #1202B	1 vial
Albumin **Std. C**, 0.5 ml, 6.0 ug/ml, #1202C	1 vial
Albumin **Std. D**, 0.5 ml, 25.0 ug/ml, #1202D	1 vial
Albumin **Std. E**, 0.5 ml, 100.0 ug/ml, #1202E	1 vial
Albumin **Std. F**, 0.5 ml, 400.0 ug/ml, #1202F	1 vial
Albumin **Positive** & **Negative Controls** (exact value printed on the vial) 0.5 ml/vial (2 vials)	
Sample Diluent 20 ml, #1203	1 bottle
Anti-Albumin-**HRP Conjugate**, 15 ml, #1204	1 bottle
HRP substrate Solution, 15 ml # TMB1200	1 bottle
Wash buffer (50X), 20 ml, dilute 1:50 with distilled water #W-50	1 bottle
Stop solution (ready-to-use), 15 ml, #ST-10	1 bottle
Complete Instruction Manual	M-1200

Intended Use:

ADI's Micro-albumin ELISA kit is intended for Quantitative Determination of Human Albumin In Urine. **For In Vitro Research Use Only (RUO).**

Introduction

The analytical determination of the protein albumin in urine is important because increased values indicate an increased risk of developing end-stage renal diseases and cardiovascular disease among people with diabetes(1,2). Also albumin in urine is a sensitive indicator of renal damage caused by exposure to nephrotoxic substances(3,4,5). The most significant and well-documented of these abnormalities is a subtle increase in the urinary albumin excretion rate, known as mico-albuminuria. Mico-albuminuria is not measurable by conventional techniques for detecting proteinuria. It is believed that mico-albuminuria represents a reversible stage of renal dysfunction, whereas overt proteinuria reflects irreversible disease. Proteinuria typically appears about twenty years after the onset of diabetes, whereas mico-albuminuria can be detected within the first ten years. Mico-albuminuria (30-150 ug/min) has been established as a marker predictive of subsequent development of diabetic nephropathy. Periodic monitoring(2-3 times/year) of urinary of albumin levels in the diabetic patient is therefore recommended so that the initial escalation of renal damage can be detected and appropriate treatment regimens can be instituted. Radial immunodiffusion, immunoturbidimetric, immunophelometric method and RIA have been used for the albumin assay in urine. ADI's Micro-albumin Quantitative using microwell competitive ELISA method provides a convenient, sensitive and specific assay for albumin and free of interference from urine specimens.

PERFORMANCE CHARACTERISTICS

1. DETECTION LIMIT

Based on sixteen replicates determinations of the zero standard, the minimum albumin concentration detectable using this assay is 0.5 ug/ml. The detection limit is defined as the value deviating by 2 SD from the zero standard.

1. PRECISION

Intra-assay precision:

	Pool A	Pool B	Pool C
Mean (ug/ml)	25.2	50.9	80.2
C.V (%)	5.3	3.3	3.6

Inter-assay precision:

	Pool A	Pool B	Pool C
Mean (ug/ml)	24.8	50.1	78.6
C.V (%)	4.2	5.1	2.9

2. RECOVERY

A known amount of human albumin (5, 10, and 20 ug/ml) was added to five patient urine samples (with original albumin concentrations of 2.6, 5.4, 12.7, 34, and 75 ug/ml) and the total albumin concentrations measured. The assay showed excellent mean recoveries of about 98% (range 88-118%).

3. LINEARITY

Five different patient urine samples (with original albumin concentrations of 50, 77.6, 103, 176, and 327 mg/l) were diluted (1:2, 1:5, and 1:10) with the zero standard and their final albumin values determined. The samples showed excellent mean recoveries of about 94% (range 85-109%).

4. SPECIFICITY

Antibodies used in this kit are highly specific for albumin with no reactivity with other serum protein. The addition of the following compound to urine samples does not interfere with the measuring of albumin.

Glucose	40 ug/ml	Creatine	10 mg/ml
Ascorbic acid	2 mg/ml	Uric acid	1.5 mg/ml
Transferrin	30 ug/ml		
Retinol binding protein	1 mg/ml		

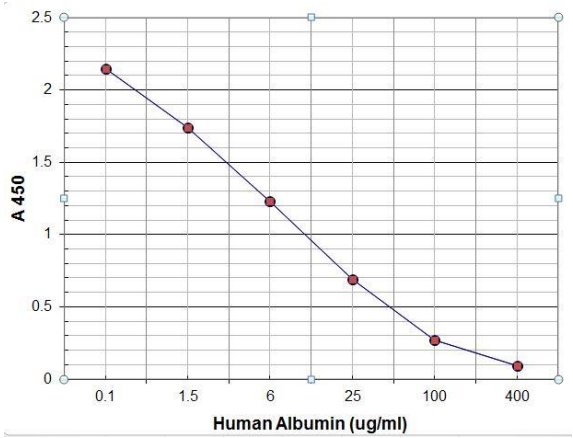
5. SPECIES CROSSREACTIVITY

Albumin from rat, bovine, chicken ovalbumin, rabbit, and human IgG also do not interfere with the assay. Other species not tested.

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Mean A_{450nm}	Calculated Concn (ug/ml)
A1, A2	**Std. A** (0 ug/ml)	2.152	
B1, B2	**Std. B** (1.5 ug/ml)	1.747	
C1, C2	**Std. C** (6.0 ug/ml)	1.233	
D1, D2	**Std. D** (25 ug/ml)	0.694	
E1, E2	**Std. E** (100 ug/ml)	0.272	
F1, F2	**Std. F** (400 ug/ml)	0.094	

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.



A typical std. assay curve (do not use this for calculating sample values)

CALCULATION OF RESULTS

Calculate the mean absorbance for each duplicate. Draw the standard curve on a lin-log graph paper by plotting absorbance values of standards against appropriate albumin concentrations. Read off the albumin concentrations of the control and patient samples.

PRINCIPLE OF THE TEST

Albumin ELISA kit is based on competitive binding of human albumin from urine samples and albumin coated on the microwell plate to the enzyme labeled anti-human albumin antibody. Higher concentrations of albumin in the urine samples result in decreased 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 **inversely** proportional to the amount of albumin 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 albumin 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.

LIMITATIONS

1. The Alpha Diagnostic International albumin ELISA test is intended for *in vitro research* use only.
2. Samples with a pH value of <4.0 or >8.0 may yield results which are respectively too high or too low. Acidified samples are usually unsuitable for the assay. The assay should not be performed if the samples exhibit significant bacterial growth or if the patient shows signs of urinary infection.
3. Bloody specimens are unsuitable for use, even if clarified by centrifugation, since blood flow is a likely a sign of contamination.

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).

REAGENT PREPRATION

Dilute wash buffer (1:50) with distilled water (20 ml stock in total of 1-liter).
Store at 4oC.

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.

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. The whole kit stability is usually six months from the date of shipping, under appropriate storage conditions.

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).* **Dilute wash buffer (1:50) with distilled water (20 ml stock in total of 1-liter).**

Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag.

1. Label or mark the microtiter well strips to be used on the plate.

2. Pipet **20 µl of standards**, control, and urine samples into appropriate wells in *duplicate*.

3. Add **100 µl of enzyme conjugate** into each well**.** Mix gently for 5-10 seconds. Cover the plate and incubate for **30 minutes** at room temperature.

4. Aspirate and wash the wells **3 times** with 300 µl wash buffer. We recommend using an automated ELISA plate washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.

5. Dispense **100 ul TMB substrate per well**. Mix gently for 5-10 seconds.

6. Cover the plate and incubate for **15 minutes** at room temperature. Blue color develops in positive wells.

7. Stop the reaction by adding **100 µl** of stopping solution to all wells. Mix gently for 5-10 seconds. Blue color turns yellow. Read the plate at 450 nm within 30 min.

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. The unused strips should be stored in a sealed bag at 4^{o}C. Addition of the HRP substrate solution starts a kinetic reaction, which is terminated by dispensing the stopping solution. Therefore, keep the incubation time for each well the same by adding the reagents in identical sequence. Plate readers measure absorbance vertically. do not touch the bottom of the wells.

DILUTION OF SAMPLES

Urine samples should be diluted **1:10 with sample buffer** before use. 100 ul of urine sample with 900 ul of sample buffer in a polystyrene tube, mix well.
Dilute serum or plasma 1:100 with sample buffer (10 ul sample and 990 ul buffer) before use. Standards & control are ready to use & need not to be diluted.

This kit has been used to measure albumin in mice transplanted with human tumors and in media from cultured cells (see references below).

Expected Values

1. It is recommended that each laboratory must determine its own normal and abnormal range.
2. Timed overnight samples and 24-hr samples have been commonly used to study mico-albuminuria. The upper limit of urinary albumin excretion in healthy adults is approx. 26 mg/24 hr (18 ug/min and 9 ug/min in overnight samples).

   Urinary albumin samples in normal subjects

First daytime	6.72 mg/l (1.89-23.9)
Cumulated	5.33 mg/l (1.31-21.7)

   In healthy subjects, albumin is ordinarily present in urine in the low range, with sustained values greater than about 15-30 mg/l usually being regarded as abnormal.

3. Urinary albumin from 123 diabetic patients was reported with ranges 4.8-209 mg/l and mean values 46.8-61.4 mg/l.

(2) Citations of ADI's Albumin ELISA kit (see web site for updated list)

Yaccoby S, 2002 Blood, 100: 4162-4168., albumin detection in tumor transplanted mice

Kajiyama Y, 2002 Mol. Cell. Biol. 22, 6122-6130, human albumin and AFP detection by ELISA in in cultured cells Hu7 Cells grown in 5% FCS

Drobna Z, 2004, Toxicology Applied Pharmacol. 201, 166-177, human albumin detection in culture medium

Schnapp LM, 2006, Am. J. Pathol., 169: 86 – 95, albumin and beta-microglobulin

Marks DJB, 2006, Lancet, 367, 668-678, human albumin elisa