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-1810

Human Ferritin

ELISA KIT Cat. No. 1810

For Quantitative Determination of Human Ferritin In Serum

For In Vitro Research Use Only

ALPHA DIAGNOSTIC INTERNATIONAL

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Web Site: www.4adi.com

Human Ferritin ELISA KIT Cat. No. 1810

For Quantitative Determination of Human Ferritin In Serum

Kit Contents: (reagents for 96 tests)

Components	Cat. #
Anti-human Ferritin coated microwell strip plate (96 wells; 8 wells x 12 strips), #1 8 1 1	1 Plate
Ferritin **Standard A**, 0.5 ml; 0 ng/ml, #1 8 1 2 A	1 Vial
Ferritin **Standard B**, 0.5 ml (10 ng/ml), #1 8 1 2 B	1 Vial
Ferritin **Standard C**, 0.5 ml (50 ng/ml), #1 8 1 2 C	1 Vial
Ferritin **Standard D**, 0.5 ml (150 ng/ml), #1 8 1 2 D	1 Vial
Ferritin **Standard E**, 0.5 ml (400 ng/ml), #1 8 1 2 E	1 Vial
Ferritin **Standard F**, 0.5 ml (800 ng/ml), #1 8 1 2 F	1 Vial
Ferritin **Controls** in protein-buffer, **Low & High** (lot specific values printed on vials) #LC-1810, HC-1810, 0.5 ml/vial	
Anti-ferritin HRP Conjugate conc (50X); 0.6 ml ,#1803	1 Vial
Assay Buffer, 25 ml, #1804	1 bottle
HRP substrate Solution; 16 ml,#TMB-1810	1 bottle
Wash buffer (10X), 50 ml, dilute 1:10 with distilled water, # WB-10	1 bottle
Stop solution (ready-to-use), 6 ml, ST-1810	1 bottle
Complete Instruction Manual, M1810	1

Introduction

Ferritin is an iron-protein complex formed from an intracellular acceptor called apoferritin. Apoferritin is a large molecular weight 450,000 protein produced by the liver. Iron as Fe(HO)3 linked to apoferritin is then stored in the cytoplasm of the reticuloendothelial system, liver, spleen and bone marrow. Ferritin is the body's iron storage protein functioning primarily as a sight for iron storage from which iron may be mobilized in response to such stimuli such as dietary change, blood loss or pregnancy. The direct quantitation of serum ferritin offers the physician a convenient and accurate measure of total body iron stores, by means of diagnosing iron-deficiency and anemia due to such causes as inflammation and hepatic or renal disease. In addition, serum ferritin concentration may be useful in detecting iron overload, which may allow the detection of idiopathic hemachromatosis in the precirrhotic storage.

ADI's Ferritin ELISA kit provides for the measurement of Ferritin in human serum.

PERFORMANCE CHARACTERISTICS

1. DETECTION LIMIT

Based on twenty replicate determinations of the zero standard, the minimum concentration of human Ferritin detected using this assay is 7..5 ng/ml. The detection limit is defined as the value deviating by 2 SD from the zero standard.

2. PRECISION

Intra-assay precision:

Sample	Mean (ng/ml)	**SD**	**CV%**
1	172.21	6.69	3.9
2	417.23	34.18	8.2
3	923.89	91.20	9.9

Inter-assay precision:

Sample	Mean (ng/ml)	**SD**	**CV%**
1	92.12	6.53	7.1
2	322.73	8.14	2.5
3	1704.63	67.01	3.9

3. RECOVERY

A known amount of Ferritin was added to three patient sera (with original Ferritin concentrations of 52 ng/ml) and the total Ferritin concentrations measured. The assay showed excellent mean recoveries of about 93-103%.

4. SPECIFICITY

The specificity of Ferritin kit was determined by measuring interference from high concentrations of human serum proteins (100 ug/dl, transferring; 1000 ug/dl, Ferric chloride, and 12 g/dl of albumin). No significant cross reactivity was observed.

Species crossreactivity

Human Ferritin ELISA kit has no significant reactivity with sera from mouse, rat or monkey when tested as human serum. Other species not tested.

General References: Clegg GA et al (1980) Prog. Bioph. Mol. Biol. 36, 56; Forman Dt et al (1980) Ann. Clin. Lab. Sci. 10, 345; Addison GM et al (1972) J Clin. Pathol. 25, 326; Halliday JW et al (1977) Lancet 621; MareschalTJC et al (1980) Clin Chim. Acta. pp 99-103; Prieto JM et al (195) Gastroenterol. 68, 525

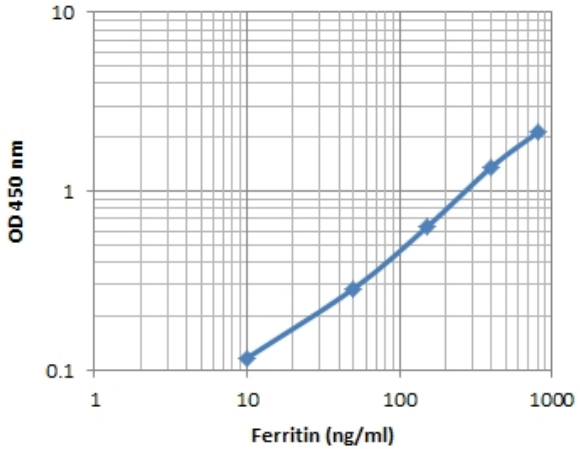
(2) Citations of ADI's ELISA kit #1810 (see web site for updated list)

Carlyon JA, 2005 Infect. Immun., 73: 7629 – 7636, ferritin in cell lysate
Theurl I, 2006, Blood 107: 4142 – 4148, ferritin concentration in cellular monocyte extracts
Shackelford RE, 2004, DNA Repair 3, 1263-1272, AT22 human lung cultured cells

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples (ng/ml)	Mean $A_{450\ nm}$	Calculated Conc. (ng/ml)
A1, A2	**Std. A** (0 ng/ml)	0.073	
B1, B2	**Std. B** (15 ng/ml)	0.116	
C1, C2	**Std. C** (50 ng/ml)	0.282	
D1, D2	**Std. D** (200 ng/ml)	0.628	
E1, E2	**Std. E** (400 ng/ml)	1.366	
F1, F2	**Std. F** (800 ng/ml)	2.127	
G1, G2	**Sample 1**	0.300	57.2

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.



*Kit-spec-XL

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

CALCULATION OF RESULTS

Calculate the mean absorbance for each duplicate. Subtract the absorbance of the zero standard from the mean absorbance values of standards, control, and samples. Draw the standard curve on log-log graph paper by plotting net absorbance values of standards against appropriate Ferritin concentrations. Read off the Ferritin concentrations of the control and patient samples. If ELISA reader software is being used, we recommend 4-paramter or 5-parameter curve.

PRINCIPLE OF THE TEST

Ferritin ELISA kit is based on simultaneous binding of human Ferritin from samples to two antibodies, one immobilized on microtiter well plates, and other conjugated to the enzyme horseradish peroxidase. After a washing step, chromogenic substrate is added and colors developed. The enzymatic reaction (color) is directly proportional to the amount of Ferritin 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 Ferritin in samples and control is read off the standard curve.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (20-100 µl) and multichannel pipet with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

The Alpha Diagnostic International Ferritin ELISA test is intended for *in vitro research* use only. The reagents contain proclin-300 as preservative; necessary care should be taken when disposing solutions. The Control Serum has been prepared from human sera shown to be negative for HBsAg and HIV antibodies. Nevertheless, such tests are unable to prove the complete absence of viruses, therefore, sera should be handled with appropriate 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).

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow to clot, and separate the serum by centrifugation at room temperature. Do not heat inactivate the serum.. If sera can not be immediately assayed , these could be stored at -20^{o}C for up to six months. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum.

Reagent Preparation

Dilute wash buffer (1:10) with distilled water (50 ml stock in 450 ml). Store at 4oC.

Dilute HRP **Enzyme Conjugate conc. 1:50** with assay diluent (20 **µl** of HRP in 1 ml of Assay Buffer)

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 6 months from the date of shipping under appropriate storage conditions.

Do not expose HRP solution to strong light during storage or use. The unused portions of the standards should be frozen in suitable aliquots for long-term use. Repeated freezing and thawing is not recommended.

TEST PROCEDURE:

(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).). **Dilute wash buffer (1:10) with distilled water (50 ml stock in 450 ml).** Dilute HRP **Enzyme Conjugate conc. 1:50** with assay diluent (20 µl of HRP in 1 ml of Assay Buffer)

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

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

2. Add **200 µl of Ab-enzyme conjugate** into each well**.** Mix gently for 5-10 seconds. Cover the plate and **incubate on a plate shaker** (approx 200 rpm). for **30 minutes** at room temperature (25-28oC).

3. Aspirate and wash the wells **5 times** with 300 µl/well with 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.

4. Dispense **150 ul TMB substrate per well.** Mix gently for 5-10 seconds. Cover the plate and **incubate on a plate shaker** for **15 minutes** at room temperature. Blue color develops into standards and positive wells. Note: It is possible to change the incubation time + 5 mins so as to get the Maximum A450 =<2.5-3.00 or within the reading capability of the ELISA readers.

5. Stop the reaction by adding **50 µl of stop solution** to **all wells** at the same timed intervals as in step 6. Mix gently for 5-10 seconds. Blue color turns yellow.

6. Measure the absorbance at **450 nm** using an ELISA reader 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. 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

Serum samples containing more than 800 ng/ml Ferritin should be diluted with the zero standard (standard A) or normal saline, reassayed, and the results obtained should be multiplied by the appropriate dilution factor.

EXPECTED VALUES

It is recommended that each laboratory must determine its own normal and abnormal ranges.

A control study was performed using this kit.

Healthy normal samples (male/female)	25-283 ng/ml

Quality Control

Standards and controls must be within the acceptable range as specified in the manual. Blanks must be <0.200 and the highest stands >1.00. Any diversion of the assay conditions such as incubation time, temperature, sample volume, or plate shaking will impact the overall A450 values. All assay conditions must be adhered to for the expected values from the kit.

Comparative Studies

The ADI Direct Ferritin ELISA kit (x) was compared with a competitors coated tube RIA kit (y). The comparison of 29 serum samples yielded the following linear regression results:

y=1.03x-20.12
R2=0.97