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 Folicle 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 (toal)

#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-1230

Direct Total Iron colorimetric assay For Serum/Plasma

Cat. # 1230 (96 tests)
Cat. # 1230-5 (480 tests)

For Quantitative Determination of total iron serum or plasma (applicable to all species)

For In Vitro Research Use Only

ALPHA DIAGNOSTIC INTERNATIONAL

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LIFE TECH Life Technologies™ Your Molecular & Cell Technology Partner

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

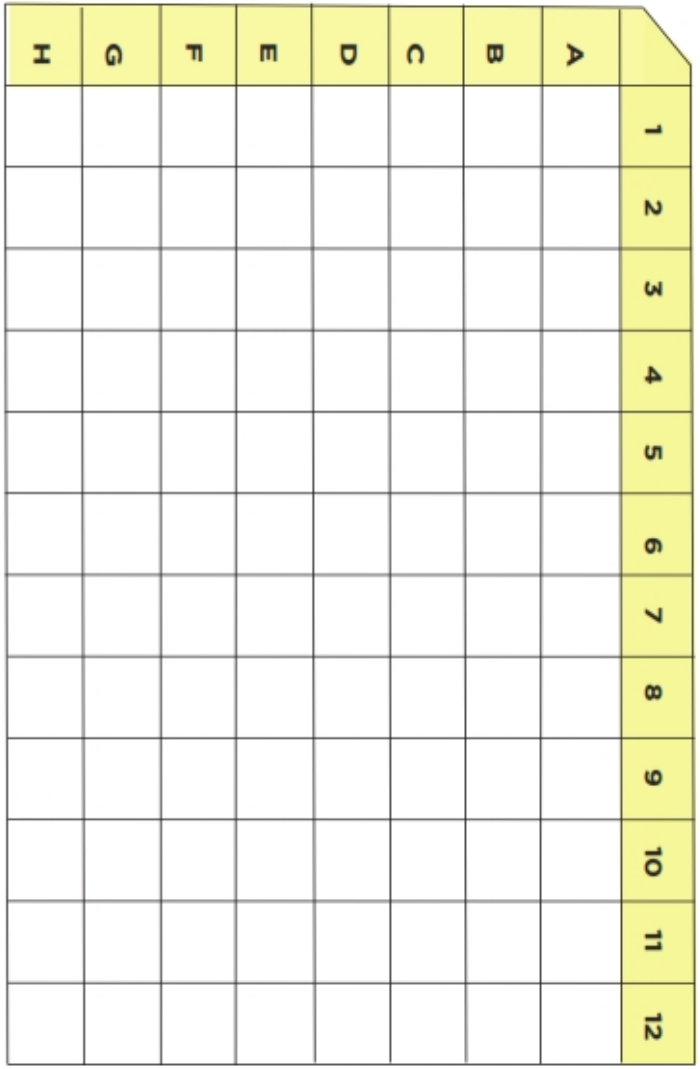
ELISA KIT # 1230 (96 tests) or 1230-5 (480 tests)
For Quantitative Determination of total iron

Kit Components	(96 tests)	(480 tests)
microwell strip plate (8 wells x 12 or (96 wells), Ready-to-use #1230P	1 plate	5 plates
Iron Stock **Standard solution** (10 mg/dL) #1230-S1	0.25 ml	1 ml
Iron **Reagent A** #1230-A	20 ml	100 ml
Iron **Reagent B** #1230-B	1 ml	4 ml
Iron **Reagent C** #1230-C	1 ml	4 ml
Complete Instruction Manual #M-1230	1	1
Store reagent A at 4oC and all other kit components at room temp. Allow to warm at room for at least 30 min prior to use.		

Introduction

Iron is a chemical element with the symbol Fe (from Latin: ferrum). Iron plays a critical role in cellular phusiology. Iron-proteins are found in all living organisms, ranging from the evolutionarily primitive archaea to humans. The color of blood is due to the hemoglobin, an iron-containing protein. As illustrated by hemoglobin, iron often is bound to cofactors, e.g. in hemes. The iron-sulfur clusters are pervasive and include nitrogenase, the enzymes responsible for biological nitrogen fixation. Iron is a necessary trace element found in nearly all living organisms. Iron-containing enzymes and proteins, often containing heme prosthetic groups, participate in many biological oxidations and in transport. Examples of proteins found in higher organisms include hemoglobin, cytochrome (see high-valent iron), and catalase. Iron level in blood is a reliable diagnostic indicator of various disease, states. Increased levels of iron concentration in blood are associated with, blood loss, increased destruction of red blood cells (e.g. hemorrhage) or decreased blood cell survival, acute hepatitis, certain sideroachrestic, anemias, ingestion of iron-rich diets, defects in iron storage (e.g., pernicious anemia). Decreased levels of blood iron may result from insufficient iron ingestion from diets, chronic blood loss pathologies, or, increased demand on iron storage as during normal pregnancy. Simple, direct and automation-ready procedures for measuring iron, concentrations find wide applications in research, drug discovery and, environmental monitoring. Iron uptake is tightly regulated by the human body, which has no regulated physiological means of excreting iron. Only small amounts of iron are lost daily due to mucosal and skin epithelial cell sloughing, so control of iron levels is mostly by regulating uptake. Regulation of iron uptake is impaired in some people as a result of a genetic defect that maps to the HLA-H gene region on chromosome 6. In these people, excessive iron intake can result in iron overload disorders, such as hemochromatosis. Many people have a genetic susceptibility to iron overload without realizing it or being aware of a family history of the problem. For this reason, it is advised that people do not take iron supplements unless they suffer from iron deficiency and have consulted a doctor. Hemochromatosis is estimated to cause disease in between 0.3 and 0.8% of Caucasians.

Iron test plate template



Total Iron Assay Kit# _____; # of test:_____

Lot #/: _____ **Expiration: _____**

Assay Date: _____

Start time:_____ End time: _____

Operator: _____

PERFORMANCE CHARACTERISTICS

1. DETECTION LIMIT

Based on twenty replicate determinations of the zero standard, the minimum concentration of iron detected using this assay is 27 ug/dL (4.8 uM). The detection limit is defined as the value deviating by 2 SD from the zero standard.

2. PRECISION

Intra-assay precision:

Three serum samples (175, 151, 82 ug/dL) were run in 20 replicates in an assay. The samples showed good intra-assay precision (4.8-7.9 %CV).

Inter-assay precision:

Three serum samples (185, 145, 73 ug/dL) were run in duplicate in ten independent assays. The samples showed good inter-assay precision (7-9.6 %CV).

3. Sample values

Serum samples from a variety of different species were tested in the assay and the total iron concn were:

Mouse Serum: 175 ± 2 ug/dL
Fetal Bovine Serum: 148 ± 2 ug/dL
Goat Serum: 88 ± 2 ug/dL
CV% <3%

4. Cross reactivity and interference

Note: (1). Iron chelators (e.g. EDTA) interfere with this assay and should be avoided in sample preparation.

(2).This kit can be applied to measure Fe^{2+} (vs. total iron) content. Prepare Working Reagent by mixing 20 vol of Reagent A, 1 vol of water and 1 vol Reagent C (no reductant in the Working Reagent). The procedure is the same as described for the total iron assay.

6. Species reactivity

Iron assay can be used in mouse, rat, human or other species.

General References: Hable ME (2006) BBRC 34, 1309-1316; WU YJ (2007) Am J. Physiol. Cell Physiol. 293, C1698-C1708; Rauf EC (2008) PNAS 105, 8591-8596

PRINCIPLE OF THE TEST

ADI's iron assay kit is designed to measure total iron directly in serum without any pretreatment. The, improved method utilizes a chromogen that forms a blue colored complex, specifically with Fe, 2+, Fe, 3+, in the sample is reduced to Fe, 2+, thus allowing, the assay for total iron concentration. The intensity of the color, measured at 590nm, is directly proportional to the iron concentration in the sample., The optimized formulation substantially reduces interference by , substances in the raw samples. , ,

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (50-200 ul) and multichannel pipet with disposable plastic tips, Reagent troughs, plate washer (recommended) and ELISA plate Reader capable of reading at 590nm..

PRECAUTIONS

The Alpha Diagnostic International total iron test is intended for *in vitro research* use only. The reagents contains no-hazardous chemicals other than common laboratory chemicals. Nevertheless, care should be taken and all reagents used and disposed with appropriate precautions. See applicable MSDS.

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. EDTA is interferes in the test and it should be avoided. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum.

All samples should be centrifuged for 15 minutes at 14,000 rpm in an Eppendorf type centrifuge prior to running in the assay.

Hemolyzed or lipemic samples should not be used with this kit. ADI also have Hemoglobin Detection kit for measuring Hb levels.

Preparation of the Working Iron reagent:

Working iron reagents should be fresh before the assay by mixing Reagents A, B, and C in the ratio of 20:1:1:

Example: 20 ml of reagent A
1 ml of reagent B
1 ml of reagent C
Total volume: 22 ml

Need 200 ul/well or ~20 ml per 96 tests. Prepare the working reagents as per requirement. Prepare fresh working reagent and do not store working reagent.

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at room temperature until the expiration date printed on the label. Reagent A should be stored at 4oC. The whole kit stability is usually 12 months from the date of shipping under appropriate storage conditions. Do not expose these solutions to strong light during storage or use.

TEST PROCEDURE - *ALLOW **ALL** REAGENTS TO REACH ROOM TEMPERATURE (25-30oC) BEFORE USE. Addition of cold reagents will reduce reaction rate and less color.*

Preparation of Working standards

First Std A is prepared from the stock by dilution 1:10 with water and then it is further diluted 2-fold for other stds B-F using the following scheme. The final can be esily scaled up if more volume of the standards is needed.

Working Stds	Stock Std @100 mg/dL	Water (ul)	Total Volume (ul)	Iron Final Conc (ug/dL)
Std. A	50 ul of Stock Std.	450 ul	450 ul	1000
Std B	80 ul of Std A	20 ul	100 ul	800
Std C	60 ul of Std A	40 ul	100 ul	600
Std D	40 ul of Std A	60 ul	100 ul	400
Std E	20 ul of Std A	80 ul	100 ul	200
Std F	10 ul of Std A	90 ul	100 ul	100
Blank	-	200 ul	200 ul	0
Note: Do not store working standards for more than 1-day and prepare fresh working standards from the stock. Need 50 ul of each std in duplicate or 100 ul total per assay.				

1. Remove required # strips and arrange them on the ELISA frame. unused strips can be stored in the supplied plastic bag. The ELISA plate frame can be saved after the test to be used again if partial plate was used for the assay. It is highly recommended to use the stds/sample layout sheet.

2. Pipet **50 ul of blank, working standards A-F, serum samples** into appropriate wells in *duplicate*.

3. Add **200 ul of assay diluent** into each well. Mix gently for 5-10 seconds. It is recommended to use a repeater pipette or mutliwell pipette to quick dispense reagent.

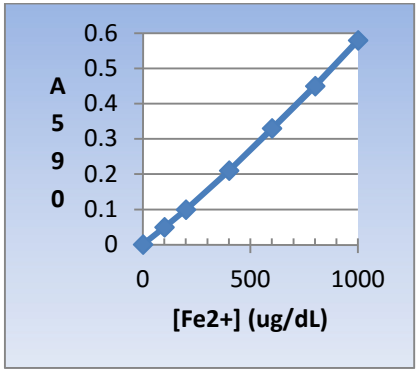
4. Incubate the plate for **40 minutes** at room temperature.

5. Read the plate at 590nm (570-630 range can be used if 590nm filter is not available but highest absorbance will be obtained at 590nm.

WORKSHEET OF TYPICAL Total Iron ASSAY

Wells	Stds/samples (ug/dL)	A_{590nm}	Calculated Conc. (ug/ml)
A1, A2	**Std. A** (1000.0)	0.58	
B1, B2	**Std. B** (800)	0.45	
C1, C2	**Std. C** (600)	0.33	
D1, D2	**Std. D** (400	0.21	
D1, D2	**Std. E** (200)	0.10	
D1, D2	**Std. F** (100)	0.05	
D1, D2	**blank** (0)	0.001	
G1, G2	**Sample 1**	0.21	398

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 standard assay curve (do not use this for calculating sample values)

Calculation of Results

Subtract the average A590 of the blanks from the standards/samples and plot the A590 versus the total iron concentration of the standards. Generate a linear regression line and use the equation, y=mx+b (y=Average delta OD; x=iron Concentration: m=slope and b= intercept) to calculate the concentrations in the unknown samples.

Conversions: 1 mg/dL Fe equals 179 µM, 0.001% or 10 ppm.