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)
#1220 Human Total Thyroxine (STFR) **#1110** Human Free STFR (fSTFR)
#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-1220

Human soluble Transferrin Receptor (sTfR) ELISA Kit

Cat. No. 1220, 96 Tests

For Quantitative Determination of sTfR In Human Serum/Plasma

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

ELISA KIT Cat. No. 1220 (96 tests)

For Quantitative Determination of human sTfR in Serum/Plasma

Kit Components (96 tests)	Qty #
Anti-Human sTfR Coated Strip plate (96 wells) #1221	1 plate
Human sTfR Std. A, 0.1 ml; 0.05 ug/ml #1222A	1 vial
Human sTfR Std. B, 0.1 ml; 0.10 ug/ml #1222B	1 vial
Human sTfR Std. C, 0.1 ml; 0.20 ug/ml #1222C	1 vial
Human sTfR Std. D, 0.1 ml; 0.50 ug/ml #1222D	1 vial
Human sTfR Std. E, 0.1 ml; 1.0 ug/ml #1222E	1 vial
Human sTfR Std. F, 0.1 ml; 2.0 ug/ml #1222F	1 vial
Dilution buffer, 13 ml, #1226DB	2 bottles
sTfR Quality Control (high), 0.05 ml, #1225HC	1 vial
sTfR Quality Control (Low), 0.05 ml, #1225LC	1 vial
Anti-human sTfR antibody-HRP Conj, 13 ml #1225	1 bottle
Wash Buffer Conc. (10X), 100 ml #1220WB	1 bottle
TMB (HRP Substrate) Soln, 13 ml #1220TM	1 bottle
Stop Solution (diluted acid), 13 ml #1220SS	1 bottle
Complete Instruction Manual # M-1220	1 Manual

Introduction

Transferrin receptor (TfR) is a carrier protein for transferrin. It is needed for the import of iron into the cell and is regulated in response to intracellular iron concentration. It imports iron by internalizing the transferrin-iron complex through receptor-mediated endocytosis.

The Transferrin receptor (TfR) is the gateway for transferrin-bound-iron entering all body cells. TfR is abundant on the surface of many newly formed cells, but the erythroid marrow cells account for 70 to 80 % of the total body TfR content. The soluble (or serum) transferrin receptor (sTfR) is a circulating truncated form of the membrane receptor protein; it is an 85 kDa glycoprotein forming in serum a 320 kDa complex with diferric transferrin. The serum sTfR concentration reflects the total body mass of cellular transferrin receptor. Anaemias associated with enhanced erythropoiesis and iron deficiency result in an elevation in the sTfR values. The normal sTfR concentrations are about 1.0 – 2.9 µg/ml for adults, when using this assay, the iron deficiency may increase the values up to 20 fold (various normal values have been established by other producers for their assays). Elevation of the soluble transferrin receptor may be also caused by haemolytic anaemia, polycythaemia and thalassemia while aplastic anaemia and chronic renal failure may result in decrease. The most important clinical use of the sTfR determination is in the differential diagnosis between iron deficiency anaemia and the anaemia of chronic disease.

2. PRECISION

Intra-assay precision:

Three serum samples (mean total sTfR concentrations 2.18 and 3.40 ug/ml) were run in 8 times. The samples showed good intra-assay precision with %CV of 3.5-4.5.
Inter-assay precision:

Two serum samples (1.76 and 4.42 ug/ml) were run in duplicate in 8 independent assays. The samples showed good inter-assay precision (3.8-4.3% CV).

3. RECOVERY

A known amount of total sTfR was added to sera (with original total sTfR concentrations of 1.15 and 1.85 ug/ml) and the final total sTfR concentration measured. The assay showed good mean recoveries of about 93% (range 91-102%).

4. LINEARITY

Two serum sample containing 2.93 and 8.16 ug/ml were further serially diluted and the sTfR recoveries were compared with the expected concentration. The samples showed excellent mean recoveries of about 102% (range 98-106%).

5. CORRELATIVE STUDY

ADI total sTfR ELISA kit was compared with a commercial immuno-turbidimetric assay (IT). Thirty-four samples (0.1-6 ug/ml) showed good correlation between the two assays.

ELISA = 1.271 IT + 0.030, R2 = 0.940, n = 34

6. SPECIES REACTIVITY

Antibodies against human sTfR are specific for human and no significant cross reactivity is observed with bovine, cat, dog, hamster, horse, mouse, rat, rabbit, and sheep sTfR. Whereas goat, monkey and pig showed reactivity.

7. Effect of Sample Matrix

Citrate, EDTA and heparin plasmas were compared to respective serum samples obtained from healthy persons (n = 10) in the same time.

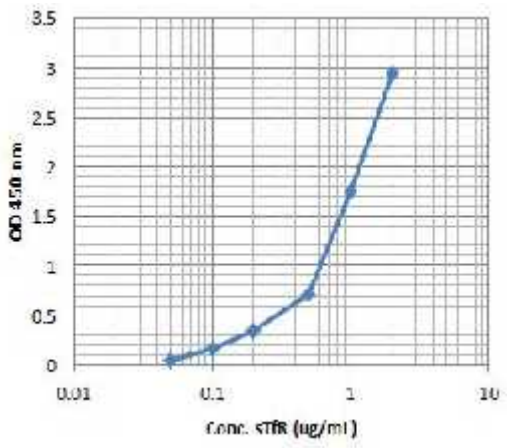
	sTfR concentration (ug/ml)			
Samples	**Serum**	**Plasma**		
		Heparin	Citrate	EDTA
1.	2.0	2.3	1.8	1.8
2.	1.7	1.6	1.6	1.6
3.	14.0	19.0	13.6	16.4
4.	11.7	11.6	9.1	8.7
5.	6.0	6.0	4.7	5.2
6.	3.0	3.2	2.8	3.2
7.	5.1	4.7	4.1	4.6
8.	2.9	3.4	2.2	2.9
9.	6.3	6.0	5.5	5.8
10.	6.4	6.4	5.6	6.1
Mean sTfR (ug/ml)	**5.9**	**6.4**	**5.1**	**5.6**
Mean plasma/serum		**108.4%**	**86.6%**	**95.3%**
Correlation coeff. R		**0.973**	**0.986**	**0.955**

General References: Raya G (2001) Clin. Chem. Lab. med. 39, 1162; Cotton F (2000) Clin. Biochem. 33, 263; Cook JD (1999) Am. J. Med. Sci. 318, 269

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Mean $A_{450\ nm}$	Calculated Concn (ug ml)
A1, A2	**Std. A** (0.05 ug/ml)	0.050	
B1, B2	**Std. B** (0.10 ug/ml)	0.170	
C1, C2	**Std. C** (0.2 ug/ml)	0.350	
D1, D2	**Std. D** (0.5 ug/ml)	0.710	
E1, E2	**Std. E** (1.0 ug/ml)	1.75	
F1, F2	**Std. F** (2.0 ug/ml)	2.950	
G1, G2	**Sample 1**	0.720	0.501 ug/ml

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values.



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

CALCULATION OF RESULTS

Most microtiter plate readers perform automatic calculations of analyte concentration. The calibration curve is constructed by plotting the absorbance (Y) of standards versus log of the known concentration (X) of standards, using the four-parameter function. Results are reported as concentration of sTfR (ug/ml) in samples. Alternatively, the logit log function can be used to linearize the calibration curve (i.e. logit of absorbance (Y) is plotted versus log of the known concentration (X) of standards).

As the standards are to be diluted 10-times, while the samples 50-times, the raw values calculated from the calibration curve have to be multiplied by a dilution factor of 5 to obtain the true results.

PERFORMANCE CHARACTERISTICS

1. Assay Sensitivity

The limit of detection (LOD) is 2ng/ml.

PRINCIPLE OF THE TEST

Human sTfR ELISA kit is based on binding of sTfR from samples specific antibodies immobilized on microtiter well plates and its detection by another antibody coupled to HRP (sandwich ELISA). After a washing step, chromogenic substrate is added and color developed. The enzymatic reaction (blue color) is directly proportional to the amount of sTfR present in the sample. The reaction is terminated by adding stopping solution (converts blue to yellow). Absorbance (450nm) is then measured on a microtiter well ELISA reader at 450 nm. and the concentration of sTfR in samples and control is read off the standard curve.

MATERIALS AND EQUIPMENT REQUIRED

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

PRECAUTIONS

The Alpha Diagnostic International sTfR 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).

SAMPLE COLLECTION AND HANDLING

The kit measures sTfR in serum and plasma (EDTA, citrate, heparin).
Samples should be assayed immediately after collection or should be stored at -20°C. Mix thoroughly thawed samples just prior to the assay and avoid repeated freeze/thaw cycles, which may cause erroneous results. Avoid using hemolyzed or lipemic samples.

Dilute samples 50x with Dilution Buffer just prior to the assay, e.g. 5 ul of sample + 245 ul of Dilution Buffer for duplicates. Mix well (not to foam). Vortex is recommended.

Stability and storage: Samples should be stored at -20°, or preferably at -70°C for long-term storage. Avoid repeated freeze/ thaw cycles. Do not store the diluted samples.

REAGENTS PREPARATION

Dilute wash buffer 1:10 with distilled water (dilute 100 ml stock in 900 ml water). Store unused buffer at 2-4oC.

Human sTfR Standards
Dilute each concentration of Standard 10x with Dilution Buffer just prior to the assay, e.g. 30 ul of Standard + 270 ul of Dilution Buffer for duplicates. Mix Standards well (we recommend vortex) before taking the desired amount from the tube as well as after adding it to the Dilution Buffer (not to foam).

Stability and storage: Opened Standards are stable 3 months when stored at 2-8°C.
Do not store the diluted Standard solutions.

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8°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. The unused portions of the standards should be stored at 2-8°C or stored frozen in small aliquots.

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).*

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 **100 ul of dilution buffer (=blank), standards**, control, and serum samples (diluted) into appropriate wells in *duplicate*. Gently mix the wells for 5-10 secs and incubate the plate at 30°C±5°C on an orbital shaker for **1 hour**. Failure to incubate at 25-30°C or shake the plate will result into lower A450.

3. Aspirate and **wash the wells 2 times** with 300 ul of 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. Add **100 ul of enzyme conjugate** into each well**.** Gently mix the wells for 5-10 secs and incubate the plate at 30°C±5°C on an orbital shaker for 1 hour. Cover the plate and incubate for **60 minutes** at room temperature.

5. Aspirate and **wash the wells 3 times** with 300 ul of 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.

6. Add **100 ul of HRP-substrate** solution into all wells. Cover the plate with aluminum foil and Mix gently for 5-10 seconds and incubate at room temp (20-30°C). Shaking is not required. Blue color develops in standards and samples. Note: incubation time may be adjusted 5-10 mins to decrease or increases total color (do not exceed A450 >3.0)

7. Stop the reaction by adding **100 ul 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.

8. Measure the **absorbance at 450 nm** using an ELISA reader within 5-10 min.

Limitations

Serum samples containing more than 2 ug/ml sTfR should be diluted with the zero standard (dilution buffer) and the results obtained should be multiplied by the appropriate dilution factor.

Performing the 25-30°C incubation temperature is crucial in order to obtain valuable results. The results obtained under different temperature can be different from the true values.

Calibrators and controls from other manufacture should not be used as they may contain serum preservatives incompatible with ADI's ELISA reagents. Whenever laboratory data conflicts with clinical findings or impressions, clinical judgment should be exercised and additional evaluations undertaken.

EXPECTED VALUES

sTfR concentration in patient sera was plotted versus Fe concentration. Average sTfR concentration of 2.08 ug/ml was found in the group of patients having normal Fe level (>10 uM), Range of the normal sTfR values were calculated as 0.9-3.3 ug/ml. Average sTfR concentration of 3.21 ug/ml was found in the group of patients having low Fe level (<10 uM).

Quality Controls

HIGH, LOW Refer to the Certificate of Analysis for current Quality Control concentration!!! Dilute Quality Control (HIGH and LOW) 50x with Dilution Buffer just prior to the assay, e.g. 5 µl of Quality Control + 245 µl of Dilution Buffer for duplicates. Mix Controls well (we recommend vortex) before taking the desired amount from the tube as well as after adding it to the Dilution Buffer (not to foam). Stability and storage: Opened Quality Controls are stable 3 months when stored at 2-8°C. Do not store the diluted Quality Controls.

REFERENCE RANGE

The following results were obtained when serum samples from 153 blood donors (84 men + 69 women, 20-65 years old, Caucasian) were assayed with the Human sTfR ELISA:

BMI (kg/m²)	N	Mean (µg/ml)	Median (µg/ml)	Max. (µg/ml)	Min. (µg/ml)	SD (µg/ml)	SEM (µg/ml)	2.5th – 97.5th percentile (µg/ml)
18-43	153	0.868	0.854	1.699	0.0720	0.307	0.0248	0.378 - 1.513
31-43	35	1.010	0.928	1.575	0.631	0.261	0.0441	0.649 - 1.558
26-30	56	0.836	0.871	1.464	0.0720	0.300	0.0401	0.303 - 1.550
18-25	62	0.817	0.790	1.699	0.253	0.316	0.0401	0.401 - 1.448

The data quoted in these instructions should be used for guidance only. Each laboratory should establish its own normal and pathological ranges for sTfR levels.