AccuDiag™ (sTfR) Soluble Transferrin Receptor ELISA Kit

REF 3126-15

IVD See External Label 2°C–8°C Σ 96 Tests

(sTfR) Soluble Transferrin Receptor ELISA	
Method	Enzyme Linked Immunosorbent Assay
Principle	Biotin-streptavidin complex
Detection Range	8.7-28.1 nmol/L
Sample	10 µL serum/plasma
Incubation Time	90 minutes
Specificity	95%
Sensitivity	0.055 nmol/L
Shelf Life	12 Months from the manufacturing date

PRODUCT FEATURES

- Very easy to use with little training
- Highly specific and consistent Assay
- Provides accurate results quickly
- Reading of results both visually and as absorbance data

INTENDED USE

Diagnostic Automation, Inc. the quantitative determination of sTfR concentration in human serum or plasma by a microplate enzyme immunoassay, Colorimetric

SUMMARY AND EXPLANATION

The Soluble Transferrin Receptor (sTfR) has been introduced as a promising new diagnostic tool for differentiating between iron deficiency anemia (IDA) and anemia of chronic disease (ACD). The circulating sTfR concentration is proportional to cellular expression of the membrane-associated TfR and increases with increased cellular iron needs and cellular proliferation. Furthermore, because serum ferritin reflects the storage iron compartment and sTfR reflects the functional iron compartment, the sTfR/log ferritin index (sTfR-F-index), based on these two values, and has been suggested as a good estimate of body iron compared with the sTfR/ferrin ratio. Distinguishing between IDA and ACD is a key step for determining whether iron supplementation would be beneficial.

As well as helping identify iron deficiency, sTfR is useful for monitoring erythropoiesis in malignancy and chronic renal disease. Development of erythropoiesis following bone marrow or stem cell transplantation is determined by the overall marrow proliferative capacity, which can be monitored with sTfR. During the aplastic period prior to transplantation, sTfR levels decline. Once erythropoiesis has recovered, sTfR levels return to normal values. In anemia of chronic renal failure, the early increase in sTfR values starting recombinant human erythropoietin therapy is useful for predicting and assessing hematologic response to therapy. The change in sTfR levels occurs well before any change in hematocrit or hemoglobin values can be detected, allowing early adjustments in erythropoietin dosage and iron supplementation therapy.

sTfR and the sTfR/ferritin ratio also appear to have other useful application. A recent study indicated that the sTfR/ferritin ratio can discriminate between anemic patients with and without celiac disease. Subclinical iron deficiency in early pregnancy is strongly associated with bacterial vaginosis, and therefore sTfR and the sTfR/log ferritin index also might have a role in highlighting the risk of this condition. The sTfR/log ferritin index has also been shown to be superior to routine tests for predicting the response to iron therapy in long-term hemodialysis patients, and in discriminating between patients with iron deficiency and various hemoglobin-nopathies. Serum sTfR also reflects the rate of erythroid proliferation and iron demand but as sTfR is not an acute phase reactant it is unaffected by inflammatory process. sTfR is therefore a useful addition to the existing anemia assays.

ASSAY PRINCIPLE

Immunoenzymometric sequential assay (TYPE 4):

The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, **in excess**, and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-sTfR antibody.

Upon mixing monoclonal biotinylated antibody, and a serum containing the native antigen, a reaction results between the native antigen and the antibody, forming an antibody-antigen complex. The interaction is illustrated by the following equation:

$$Ag_{(sTfR)} + {}^{Btn}Ab_{(m)} \underset{k_{-a}}{\overset{k^{a}}{\rightleftharpoons}} Ag_{(sTfR)} - {}^{Btn}Ab_{(m)}$$

${}^{Btn}Ab_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity).
$Ag_{(sTfR)}$ = Native Antigen (Variable Quantity).
$Ag_{(sTfR)} - {}^{Btn}Ab$ (m) Antigen-antibody complex (Variable Quantity).
K_a = Rate Constant of Association.
k_{-a} = Rate Constant of Disassociation.

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:

$$Ag_{(sTfR)} - {}^{Btn}Ab_{(m)} + Ab\ \underline{Streptavidin}_{CW} \Rightarrow \underline{Immobilized\ complex\ (IC)}$$

$\underline{Streptavidin}_{CW}$ = Streptavidin immobilized on well.
Immobilized complex (IC) = Ag-Ab bound to the well

After a suitable incubation period, the antibody-antigen bound fraction is separated from unbound antigen by decantation or aspiration. Another antibody (directed at a different epitope) labeled with an enzyme is added. Another interaction occurs to form an enzyme labeled antibody-antigen-biotinylated-antibody complex on the surface of the wells. Excess enzyme is washed off via a wash step. A suitable substrate is added to produce color measurable with the use of a microplate spectrophotometer. The enzyme activity on the well is directly proportional to the native free antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

$$(IC) + {}^{Enz}Ab_{(x\text{-}sTfR)} \underset{k_{-b}}{\overset{k_b}{\rightleftharpoons}} {}^{Enz}Ab_{(x\text{-}sTfR)} - IC$$

${}^{Enz}Ab_{(x\text{-}sTfR)}$ = Enzyme labeled Antibody (Excess Quantity)
${}^{Enz}Ab_{(x\text{-}sTfR)}$ – IC = Antigen-Antibody Complex
K_b= Rate Constant of Association.
k_{-b} = Rate Constant of Disassociation.

SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood; serum or heparanised plasma in type and taken with the usual precautions in the collection of venipuncture samples. For accurate comparison to establish normal values, a fasting morning serum sample should be obtained. The blood should be collected in a redtop veni-puncture tube with or without additives or anti-coagulants (for serum) or evacuated tube(s) containing EDTA or heparin (for plasma). Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.020ml (20 µl) of the specimen is required.

REAGENTS

Materials provided with the test kit

A. **sTfR Calibrators – 0.5 ml/vial – Icons A-F**
Six (6) vials of serum reference for sTfR at concentrations of 0 (**A**), 3.0 (**B**), 10(**C**), 20 (**D**), 40 (**E**) and 80 (**F**) in nmol/L. Store at 2-8°C. A preservative has been added.

B. **sTfR Enzyme Reagents – 12.0 ml/vial**
One (1) vial of sTfR (Analog) - horseradish peroxides (HRP) conjugate in a protein-stabilizing matrix with red dye. Store at 2-8°C.

C. **sTfR Biotin Reagent – 12.0 ml/vial**
One (1) vial of reagent contains anti-sTfR biotinylated purified rabbit IgG conjugate in buffer, blue dye and preservative. Store at 2-8°C

D. **Streptavidin Coated Plate – 96 wells**
One 96-well microplate coated with 1.0 µg/ml streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

E. **Wash Solution Concentrate – 20.0 ml/vial**
One (1) vial contains a surfactant in buffered saline. A preservative has been added. Store at 2-8 °C.

F. **Substrate Solution – 14.0 ml/vial**
One (1) bottle contains tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.

G. **Stop Solution – 8ml/vial**
One (1) vial contains a strong acid (0.5M H_2SO_4). Store at 2-8°C.

H. **Product Instructions.**

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.**
Note 3: Above reagents are for a single 96-well microplate.

Materials required but not provided

1. Pipette capable of delivering 0.010 & 0.050 ml (10 & 50 µL) with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100 & 0.350 ml (100 & 350 µL) volumes with a precision of better than 1.5 %.
3. Microplate washer or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials.

REAGENT PREPARATION

1. **Wash Buffer**
Dilute contents of wash solution to 1000ml with distilled or deionized water in a suitable storage container. Diluted buffer can be stored at 2-30°C for up to 60 days.

Note: Do not use reagents that are contaminated or have bacteria growth.

ASSAY PROCEDURE

Before proceeding with the assay, bring all reagents, reference calibrators and controls to room temperature (20-27°C).

****Test procedure should be performed by a skilled individual or trained professional****

1. Format the microplates' wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate.
Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.
2. Pipette 0.010ml (10µL) of the appropriate serum reference calibrator, control or specimen into the assigned well.
3. Add 0.100 ml (100 µL) of the sTfR Biotin Reagent to all wells.
4. Swirl the microplate gently for 20-30 seconds to mix.
5. Cover and incubate for 45 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
7. Add 0.350ml (350 µL) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instructions for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoid air bubbles) to dispense the wash. Decant the wash repeat two (2) additional times.**
8. Add 0.100 ml (100µL) of Anti-sTfR Enzyme Reagent to all wells.

9. Cover and incubate for 30 minutes at room temperature.
10. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
11. Add 0.350 ml (350µL) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate.
12. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instructions for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoid air bubbles) to dispense the wash. Decant the wash repeat two (2) additional times.**
13. Add 0.100 ml (100µL) of substrate solution to all wells. **Always add reagents in the same order to minimize reaction time differences between wells.** DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION.
14. Incubate at room temperature for fifteen (15) minutes.
15. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds. Always add reagents in the same order to minimize reaction time differences between wells.
16. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630 nm. **The results should be read within fifteen (15) minutes of adding the stop solution.**

Note: Dilute the samples suspected of concentrations higher than 80nmol/mL with sTfR '0' nmol/mL calibrator and multiply result by dilution factor.

RESULTS

A dose response curve is used to ascertain the concentration of sTfR in unknown specimens.

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding sTfR concentration in nmol/L on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Connect the points with a best-fit curve.
4. To determine the concentration of sTfR for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve and read the concentration (in nmol/L) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance in the patient/ sample (0.978) intersects the dose response curve at 18.0 nmol/L sTfR concentration (See Figure 1).

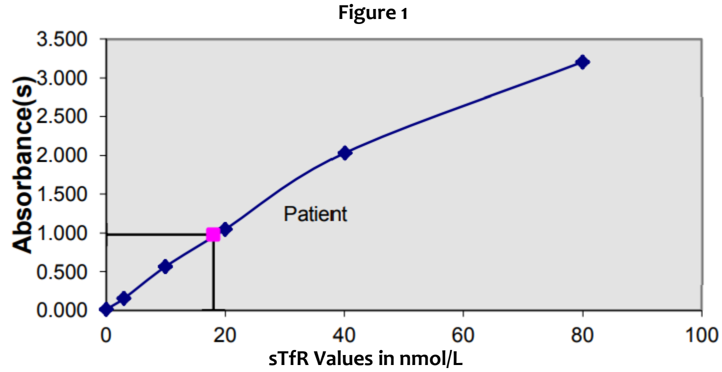
Note: Computer data reduction software designed for ELISA assay may also be used for the data reduction. **If such software is utilized, the validation of the software should be ascertained.**

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (nmol/L)
Cal A	A1	0.012	0.013	0.0
	B1	0.013		
Cal B	C1	0.156	0.156	3.0
	D1	0.157		
Cal C	E1	0.579	0.567	10.0
	F1	0.555		
Cal D	G1	1.075	1.048	20.0
	H1	1.021		
Cal E	A2	2.069	2.032	40.0
	B2	1.995		
Cal F	C2	3.240	3.204	80.0
	D2	3.168		
Pat # 1	E3	0.958	0.978	**18.0**
	F3	0.998		

**The above data and table below is for example only. Do not use it for calculating your results.

Figure 1



Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator F should be ≥ 1.3.
2. Four out of six quality control pools should be within the established ranges.

RISK ANALYSIS

The MSDS and Risk Analysis Form for this product is available on request from Diagnostic Automation, Inc.

ASSAY PERFORMANCE

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Diagnostic Automation, Inc. IFU may yield inaccurate results.
10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.

11. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
12. Risk Analysis – as required by CE Mark IVD Directive 98/79/EC – for this and other devices, made by Diagnostic Automation, can be requested via email from QC@rapidtest.com.

INTERPRETATION

1. **Measurements and interpretation of results must be performed by a skilled individual or trained professional.**
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. 'Heterophilic antibodies; a problem for all kinds of immunoassays' Clin. Chem. 1988:3427-33) For diagnostic purposes, the results from this assay should be in combination with clinical examination, patient history and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Diagnostic Automation, Inc. shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the tests, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and high range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

EXPECTED VALUES

In agreement with established reference intervals for a "normal" adult population, the expected ranges for the sTfR Microplate ELISA Test System are detailed in Table 1.

TABLE 1

Expected Values for the sTfR Test System	
Mean	Range
18.4 nmol/L	8.7 - 28.1 nmol/L

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal" persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the manufacturer only until an in- house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

PERFORMANCE CHARACTERISTICS

Precision

The within and between assay precision of the sTfR Microplate ELISA Test System were determined by analyses on three different levels of pool control sera. The number, mean values, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

TABLE 2
Within Assay Precision (Values in nmol/L)

Sample	N	X	σ	CV
Low	20	10.67	0.62	5.8
Normal	20	21.25	0.93	4.4
High	20	34.54	1.40	4.1

TABLE 3
Between Assay Precision (Values in nmol/L)

Sample	N	X	σ	CV
Low	5	11.19	0.85	7.6
Normal	5	22.07	2.25	10.2
High	5	32.47	2.03	6.3

Sensitivity

The sTfR Microplate ELISA Test System has a sensitivity of 0.055 nmol/L. The sensitivity was ascertained by determining the variability of the 0 nmol/L serum calibrator and using the 2 σ (95% certainty) statistic to calculate the minimum dose.

Specificity

The % cross reactivity of the sTfR antibody to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of sTfR needed to displace the same amount of labeled analog.

Table 4

Substance	Cross Reactivity
Human Diferrictransferrin	ND
Human Apotransferrin	ND
Human Heart Ferritin	ND
Human Liver Ferritin	ND
Human Spleen Ferritin	ND
Triolein	ND
Human Serum Albumin	ND
Bilirubin	ND
Hemoglobin	ND

PRECAUTIONS

For in Vitro Diagnostic Use.
Not for Internal or External Use in Human or Animals.

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA required tests. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

REFERENCES

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MANUFACTURER AND BRAND DETAILS

ISO 13485:2016

bsi ISO 13485 Quality Management for Medical Devices CERTIFIED

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REF 3126-15	AccuDiag™ - (sTfR) Soluble Transferrin Receptor
Brand Name	AccuDiag™
EC REP	CEpartner4U, Esdoornlaan 13, 3951 DB Maarn, The Netherlands. www.cepartner4u.eu
Revision Date: 11-04-2020	