DRG® Human Soluble Transferrin Receptor ELISA (EIA-4256)

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Please use only the valid version of the package insert provided with the kit.

This kit is intended for Research Use Only.

Not intended for diagnostic procedures

INTENDED USE

The Human sTfR ELISA is a sandwich enzyme immunoassay for measurement of human soluble transferrin receptor.

Features

- **For research use only!**
- The total assay time is less than 3 hours.
- The kit measures total soluble transferrin receptor in serum, plasma (EDTA, citrate, heparin) and tissue culture medium.
- Assay format is 96 wells.
- Quality Controls are human serum based.
- Standard is natural human blood isolated sTfR based.
- Components of the kit are provided ready to use or concentrated.

STORAGE, EXPIRATION

Store the complete kit at 2-8°C. Under these conditions, the kit is stable until the expiration date (see label on the box).
For stability of opened reagents see Chapter 9.

TEST PRINCIPLE

In the Human sTfR ELISA, standards, quality controls and samples are incubated in microplate wells pre-coated with monoclonal anti-human sTfR antibody. After 60 minutes incubation and washing, monoclonal anti-human sTfR antibody, conjugated with horseradish peroxidase (HRP) is added to the wells and incubated for 60 minutes with captured sTfR. Following another washing step, the remaining HRP conjugate is allowed to react with the substrate solution (TMB). The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured. The absorbance is proportional to the concentration of sTfR. A standard curve is constructed by plotting absorbance values against concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

PRECAUTIONS

- **For professional use only.**
- Wear gloves and laboratory coats when handling kit materials.

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- Do not drink, eat or smoke in the areas where kit materials are being handled.
- This kit contains components of human origin. These materials were found non-reactive for HBsAg, HCV antibody and for HIV 1/2 antigen and antibody. These materials should be handled as potentially infectious, as no test can guarantee the complete absence of infectious agents.
- Avoid contact with the acidic Stop Solution and Substrate Solution, which contains hydrogen peroxide and tetramethylbenzidine (TMB). Wear gloves and eye and clothing protection when handling these reagents. Stop and/or Substrate Solutions may cause skin/eyes irritation. In case of contact with the Stop Solution and the Substrate Solution wash skin/eyes thoroughly with water and seek medical attention, when necessary.
- The materials must not be pipetted by mouth.

TECHNICAL HINTS

- Reagents with different lot numbers should not be mixed.
- Use thoroughly clean glassware.
- Use deionized (distilled) water, stored in clean containers.
- Avoid any contamination among samples and reagents. For this purpose, disposable tips should be used for each sample and reagent.
- Substrate Solution should remain colourless until added to the plate. Keep Substrate Solution protected from light.
- Stop Solution should remain colourless until added to the plate. The colour developed in the wells will turn from blue to yellow immediately after the addition of the Stop Solution. Wells that are green in colour indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.
- Dispose of consumable materials and unused contents in accordance with applicable national regulatory requirements.

REAGENT SUPPLIED

Kit Components	State	Quantity
Antibody Coated Microtiter Strips	ready to use	96 wells
Conjugate Solution	ready to use	13 ml
Set of Standard	concentrated	6 x 0.1 ml
Quality Control HIGH	concentrated	0.05 ml
Quality Control LOW	concentrated	0.05 ml
Dilution Buffer	ready to use	2 x 13 ml
Wash Solution Conc. (10x)	concentrated	100 ml
Substrate Solution	ready to use	13 ml
Stop Solution	ready to use	13 ml
Product Data Sheet + Certificate of Analysis		1 pc

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MATERIAL REQUIRED BUT NOT SUPPLIED

- Deionized (distilled) water
- Test tubes for diluting samples
- Glassware (graduated cylinder and bottle) for Wash Solution (Dilution Buffer)
- Precision pipettes to deliver 5 -1000 µl with disposable tips
- Multichannel pipette to deliver 100 µl with disposable tips
- Absorbent material (e.g. paper towels) for blotting the microtiter plate after washing
- Vortex mixer
- Orbital microplate shaker capable of approximately 300 rpm
- Microplate washer (optional). [Manual washing is possible but not preferable.]
- Microplate reader with 450 ± 10 nm filter, preferably with reference wavelength 630 nm (alternatively another one from the interval 550 – 650 nm)
- Software package facilitating data generation and analysis (optional)

PREPARATION OF REAGENTS

- **All reagents need to be brought to room temperature prior to use.**
- **Warm-up the Dilution Buffer to 25-30°C prior to use.**
- **Always prepare only the appropriate quantity of reagents for your test.**
- **Do not use components after the expiration date marked on their label.**

A. Assay reagents supplied ready to use

Antibody Coated Microtiter Strips

Stability and storage:

Return the unused strips to the provided aluminium zip-sealed bag with desicant and seal carefully. Remaining Microtiter Strips are stable 3 months stored at 2-8°C and protected from the moisture.

Conjugate Solution

Dilution Buffer

Substrate Solution

Stop Solution

Stability and storage:

Opened reagents are stable 3 months when stored at 2-8°C.

B. Assay reagents supplied concentrated

Human sTfR Standards

Dilute each concentration of Standard 10x with Dilution Buffer just prior to the assay, e.g. 30 µl of Standard + 270 µl of Dilution Buffer for duplicates. Mix well (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**.

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 well (not to foam).

Stability and storage:

Opened Quality Control s are stable 3 months when stored at 2-8°C.

Do not store the diluted Quality Controls.

Wash Solution Conc. (10x)

Dilute Wash Solution Concentrate (10x) ten-fold in distilled water to prepare a 1x working solution. Example: 100 ml of Wash Solution Concentrate (10x) + 900 ml of distilled water for use of all 96-wells.

Stability and storage:

The diluted Wash Solution is stable 1 month when stored at 2-8°C. Opened Wash Solution Concentrate (10x) is stable 3 months when stored at 2-8°C.

PREPARATION OF SAMPLES

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 µl of sample + 245 µl 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.

Note: It is recommended to use a precision pipette and a careful technique to perform the dilution in order to get precise results.

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ASSAY PROCEDURE

1. Pipet **100 µl** of diluted Standards, Quality Controls, Dilution Buffer (=Blank) and samples, preferably in duplicates, into the appropriate wells. See Figure 1 for example of work sheet.
2. Incubate the plate at 30°C (±5°C) for **1 hour**, shaking at ca. 300 rpm on an orbital microplate shaker.
   Performing the incubation at the temperature of 25-35°C is crucial in order to obtain valuable results!
3. Wash the wells **3-times** with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
4. Add **100 µl** of Conjugate Solution into each well.
5. Incubate the plate at 30°C (±5°C) for **1 hour**, shaking at ca. 300 rpm on an orbital microplate shaker.
   Performing the incubation at the temperature of 25-35°C is crucial in order to obtain valuable results!
6. Wash the wells **3-times** with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
7. Add **100 µl** of Substrate Solution into each well. Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.
8. Incubate the plate for **10 minutes** at room temperature (20-30°C). The incubation time may be extended [up to 20 minutes] if the reaction temperature is below than 20°C.
   Do not shake the plate during the incubation.
9. Stop the colour development by adding **100 µl** of Stop Solution.
10. Determine the absorbance of each well using a microplate reader set to 450 nm, preferably with the reference wavelenght set to 630 nm (acceptable range: 550 – 650 nm). Subtract readings at 630 nm (550 – 650 nm) from the readings at 450 nm. The absorbance should be read within 5 minutes following step 9.

Note 1: If some samples and standard/s have absorbances above the upper limit of your microplate reader, perform a second reading at 405 nm. A new standard curve, constructed using the values measured at 405 nm, is used to determine sTfR concentration of off-scale standards and samples. The readings at 405 nm should not replace the readings for samples that were "in range" at 450 nm.

Note 2: Manual washing: Aspirate wells and pipet 0.35 ml Wash Solution into each well. Aspirate wells and repeat twice. After final wash, invert and tap the plate strongly against paper towel. Make certain that Wash Solution has been removed entirely.

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	strip 1+2	strip 3+4	strip 5+6	strip 7+8	strip 9+10	strip 11+12
A	Standard 2	Blank	Sample 8	Sample 16	Sample 24	Sample 32
B	Standard 1	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
C	Standard 0.5	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
D	Standard 0.2	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
E	Standard 0.1	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
F	Standard 0.05	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
G	QC HIGH	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
H	QC LOW	Sample 7	Sample 15	Sample 23	Sample 31	Sample 39

Figure 1: Example of a work sheet.

CALCULATIONS

Most microplate readers perform automatic calculations of analyte concentration. The standard curve is constructed by plotting the mean absorbance (Y) of Standards against the known concentration (X) of Standards in logarithmic scale, using the four-parameter algorithm. Results are reported as concentration of sTfR µg/ml in samples.

Alternatively, the logit log function can be used to linearize the standard curve i.e. logit of the mean absorbance (Y) is plotted against log of the known concentration (X) of Standards.

The measured concentrations of samples and Quality Controls calculated from the standard curve have to be multiplied by their respective dilution factor. Since samples and Quality Controls are diluted 50x while standards are diluted 10x, the ratio 50/10 = 5 have to be used as the dilution factor.

Example: 13.5 µg/ml (from standard curve) x 5 (dilution factor) = 67.5 µg/ml (real concentration in sample).

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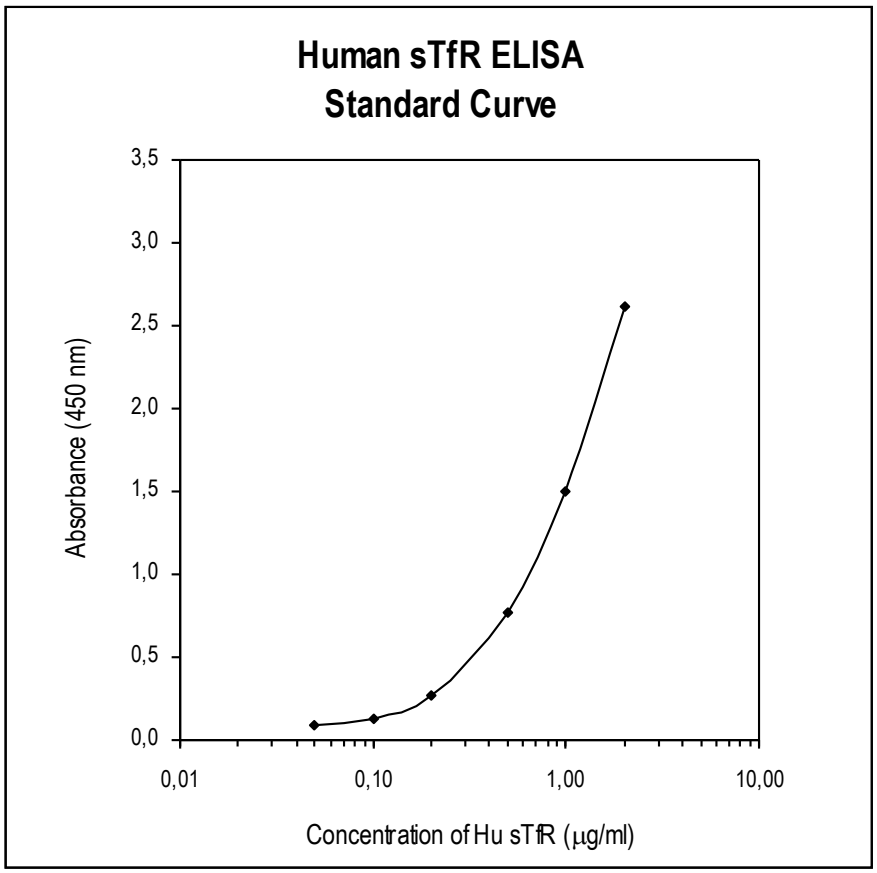

Figure 2: Typical Standard Curve for Human sTfR ELISA.

C. Effect of sample matrix

EDTA, citrate and heparin plasmas were compared to respective serum samples from the same 10 individuals. Results are shown below:

Volunteer No.	Serum *(µg/ml)*	Plasma *(µg/ml)*		
		EDTA	Citrate	Heparin
1	2.0	1.8	1.8	2.3
2	1.7	1.6	1.6	1.6
3	14.0	16.4	13.6	19.0
4	11.7	8.7	9.1	11.6
5	6.0	5.2	4.7	6.0
6	3.0	3.2	2.8	3.2
7	5.1	4.6	4.1	4.7
8	2.9	2.9	2.2	3.4
9	6.3	5.8	5.5	6.0
10	6.4	6.1	5.6	6.4
Mean (µg/ml)	**5.9**	**5.6**	**5.1**	**6.4**
Mean Plasma/Serum (%)	**-**	**94.8**	**86.3**	**108.6**
Coefficient of determination R^2	**-**	**0.96**	**0.99**	**0.97**

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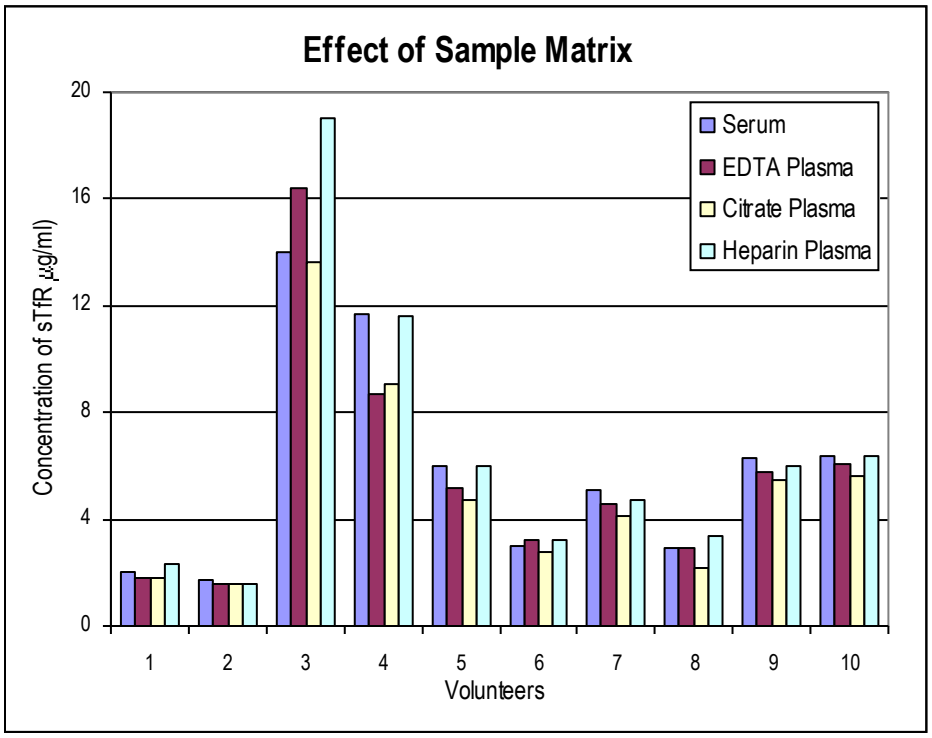

Fig. 3: sTfR levels measured using Human sTfR ELISA from 10 individuals using serum, EDTA, citrate and heparin plasma, respectively.

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DEFINITION OF THE STANDARD

The Standard used in this kit is a natural sTfR isolated from human blood.

Concentration Unit Conversions

(calculated from the sTfR molar mass):

1 nM = 0.075 µg/ml
1 µg/ml = 13.33 nM

PRELIMINARY POPULATION AND CLINICAL DATA

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

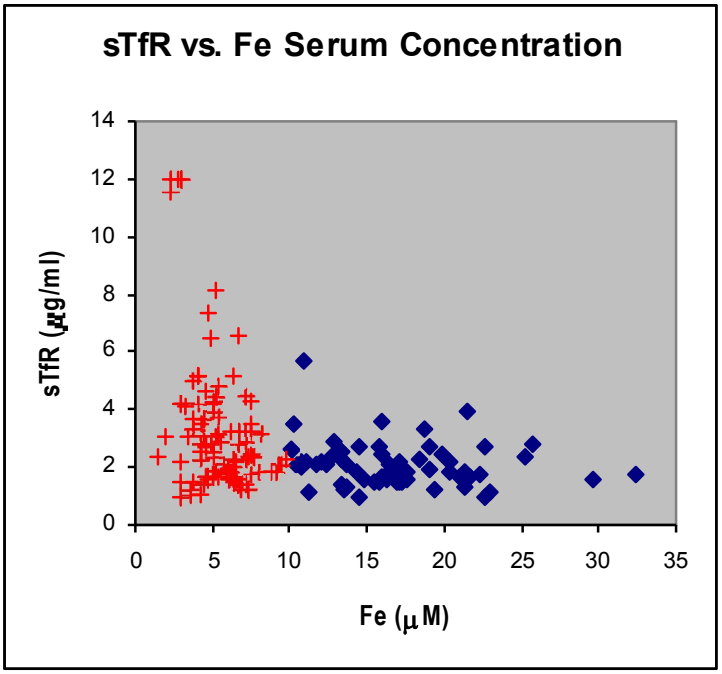

Fig. 4: sTfR vs. Fe Serum Concentration

D. Reference range

The data quoted in these instructions should be used for guidance only. It is recommended that each laboratory include its own panel of control samples in the assay. Each laboratory should establish its own normal and pathological reference ranges for sTfR levels with the assay.

METHOD COMPARISON

The Human sTfR ELISA was compared to a commercial Immunoturbidimetry (IT). The following correlation graph was obtained.

$y = 0.740x + 0.146$

$R^2 = 0.94$

$N = 34$

TROUBLESHOOTING AND FAQS

Weak signal in all wells

Possible explanations:

- Omission of a reagent or a step
- Improper preparation or storage of a reagent
- Assay performed before reagents were allowed to come to room temperature
- Improper wavelength when reading absorbance

High signal and background in all wells

Possible explanations:

- Improper or inadequate washing
- Overdeveloping; incubation time with Substrate Solution should be decreased before addition of Stop Solution
- Incubation temperature over 30°C

High coefficient of variation (CV)

Possible explanation:

- Improper or inadequate washing
- Improper mixing Standards, Quality Controls or samples

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Assay Procedure Summary