TFRC (Human) ELISA Kit

Catalog Number KA0039

96 assays

Version: 12

Intended for research use only

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Introduction

Intended Use

The TFRC (Human) ELISA Kit is a sandwich enzyme immunoassay for the quantitative 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 transferring receptor in serum and plasma (EDTA, citrate, heparin).
- ✓ Assay format is 96 wells.
- ✓ Quality Controls are human serum based. No animal sera are used.
- ✓ Standard is natural human blood isolated sTfR based.
- ✓ Components of the kit are provided ready to use or concentrated.

Background

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. 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 its 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.

Areas of investigation:
Iron metabolism

Principle of the Assay

In the TFRC (Human) ELISA Kit, 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.

General Information

Materials Supplied

List of component

Component	State	Amount
Antibody Coated Microtiter Strips	ready to use	96 wells
Conjugate Solution	ready to use	13 mL
Set of Standards	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

Storage Instruction

Store the complete kit at 2-8°C. Under these conditions, the kit is stable until the expiration date (see label on the box).

Materials Required but Not Supplied

- ✓ Deionized (distilled) water
- ✓ Test tubes for diluting samples
- ✓ Glassware (graduated cylinder and bottle) for Wash Solution
- ✓ 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 microplate 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)

Precautions for Use

- Precautions

✓ For professional use only.

✓ Wear gloves and laboratory coats when handling immunodiagnostic materials.

✓ Do not drink, eat or smoke in the areas where immunodiagnostic 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.

Assay Protocol

Reagent Preparation

- ✓ 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.

- 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.

- 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 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.

✓ 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.

Note:

Concentration of analyte in Quality Controls need not be anyhow associated with normal and/or pathological concentration in serum or another body fluid. Quality Controls serve just for control that the kit works in accordance with COA and that ELISA test was carried out properly.

- ✓ 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.

Sample Preparation

- ✓ 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.

  See Performance Characteristics for effect of sample matrix (serum/plasma) on the concentration of sTfR.

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

Assay Procedure

1. Pipet 100 µL of diluted Standards, Quality Controls, Dilution Buffer (=Blank) and samples, preferably in duplicates, into the appropriate wells. See Plate Layout for example of work sheet.
2. Incubate the plate at 25-30°C for 1 hour, shaking at ca. 300 rpm on an orbital microplate shaker. Performing the incubation at the appropriate temperature 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 25-30°C for 1 hour, shaking at ca. 300 rpm on an orbital microplate shaker.

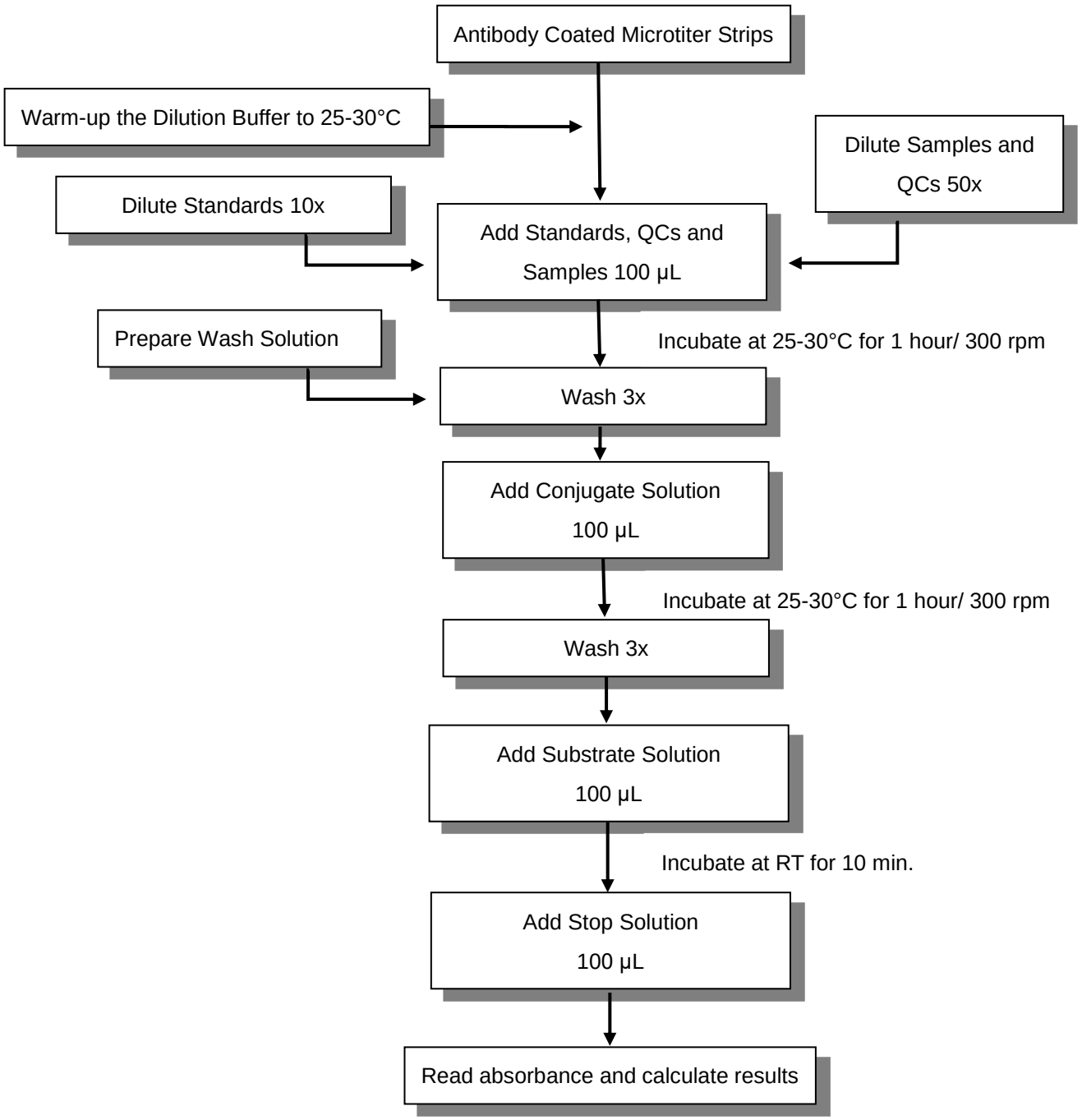
Performing the incubation at the appropriate temperature is crucial in order to obtain valuable results!

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). 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.*
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.*

- Assay Procedure Summary



Data Analysis

Calculation of Results

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: 0.4 µg/mL (from standard curve) x 5 (dilution factor) = 2.0 µg/mL (real concentration in sample).

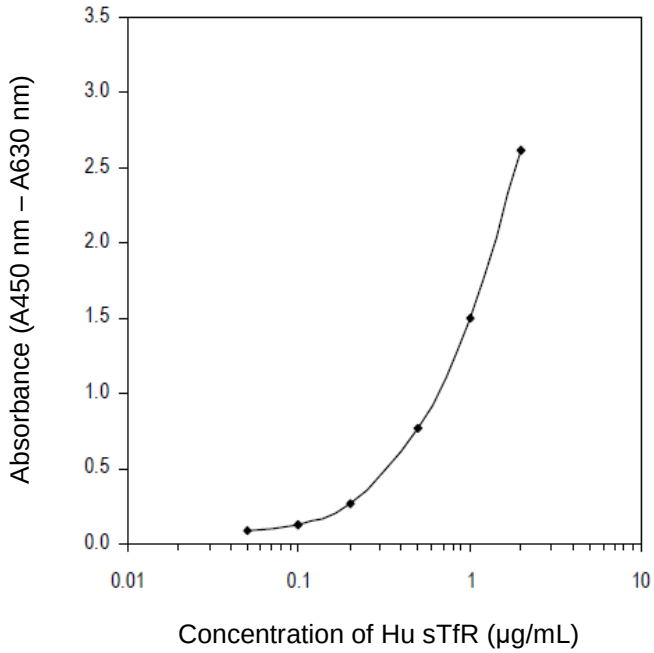


Figure 1: Typical Standard Curve for TFRC (Human) ELISA Kit

Performance Characteristics

- Sensitivity

  Limit of Detection (LOD) (defined as concentration of analyte giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: A_{blank} + 3xSD_{blank}) is calculated from the real sTfR values in wells and is 2 ng/mL.

  *Dilution Buffer is pipetted into blank wells.

- Limit of assay

  Results exceeding sTfR level 10 µg/mL should be repeated with more diluted samples (e.g. 100x). Dilution factor needs to be taken into consideration when calculating the sTfR concentration.

- Specificity

  The antibodies used in this ELISA are specific for human sTfR.

  The sTfR ELISA exhibits no interference with hemoglobin (0.1 mg/mL), bilirubin (170 µmol/L), or triglycerides (5.0 mmol/L). However, higher levels of hemoglobin can interfere with performance of this ELISA, therefore, we discourage the customers from using hemolyzed samples.

  Sera of several mammalian species were measured in the assay. See results below.

Mammalian serum sample	Observed cross activity
Bovine	no
Cat	no
Dog	no
Goat	yes
Hamster	no
Horse	no
Monkey	yes
Mouse	no
Pig	yes
Rabbit	no
Rat	no
Sheep	no

- Precision

  Intra-assay (Within-Run, n=8)

Sample	Mean (µg/mL)	SD (µg/mL)	CV (%)
1	1.27	0.04	3.4
2	5.22	0.23	4.3

  Inter-assay (Run-to-Run, n=8)

Sample	Mean (µg/mL)	SD (µg/mL)	CV (%)
1	1.51	0.11	7.0
2	6.12	0.33	5.5

- Spiking Recovery

  Serum samples were spiked with different amounts of human sTfR and assayed.

Sample	Observed (μg/mL)	Expected (μg/mL)	Recovery O/E (%)
1	0.28	-	-
	2.18	2.28	96
	1.39	1.28	109
	0.78	0.78	100
2	0.21	-	-
	2.12	2.21	96
	1.26	1.21	104
	0.68	0.71	96

- Linearity

  Serum samples (50x diluted) were serially diluted with Dilution Buffer and assayed.

Sample	Dilution	Observed (μg/mL)	Expected (μg/mL)	Recovery O/E (%)
1	-	5.13	-	-
	2x	2.43	2.57	95
	4x	1.25	1.28	98
	8x	0.66	0.64	103
2	-	5.90	-	-
	2x	2.53	2.95	86
	4x	1.39	1.48	94
	8x	0.65	0.74	88

- 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	0.89	0.61	0.66	0.84
2	1.43	1.15	1.07	1.39
3	1.33	0.80	0.92	1.25
4	1.24	1.06	0.93	1.19
5	1.19	1.03	0.90	1.38
6	1.18	0.78	0.76	1.09
7	1.54	1.16	1.14	1.54
8	2.19	1.78	1.70	2.33
9	1.35	0.84	1.08	1.34
10	0.94	0.90	0.72	1.01
Mean (µg/mL)	1.3	1.0	1.0	1.3
Mean Plasma/Serum(%)	-	76.1	74.4	100.6
Coefficient of determination R^2	-	0.82	0.97	0.95

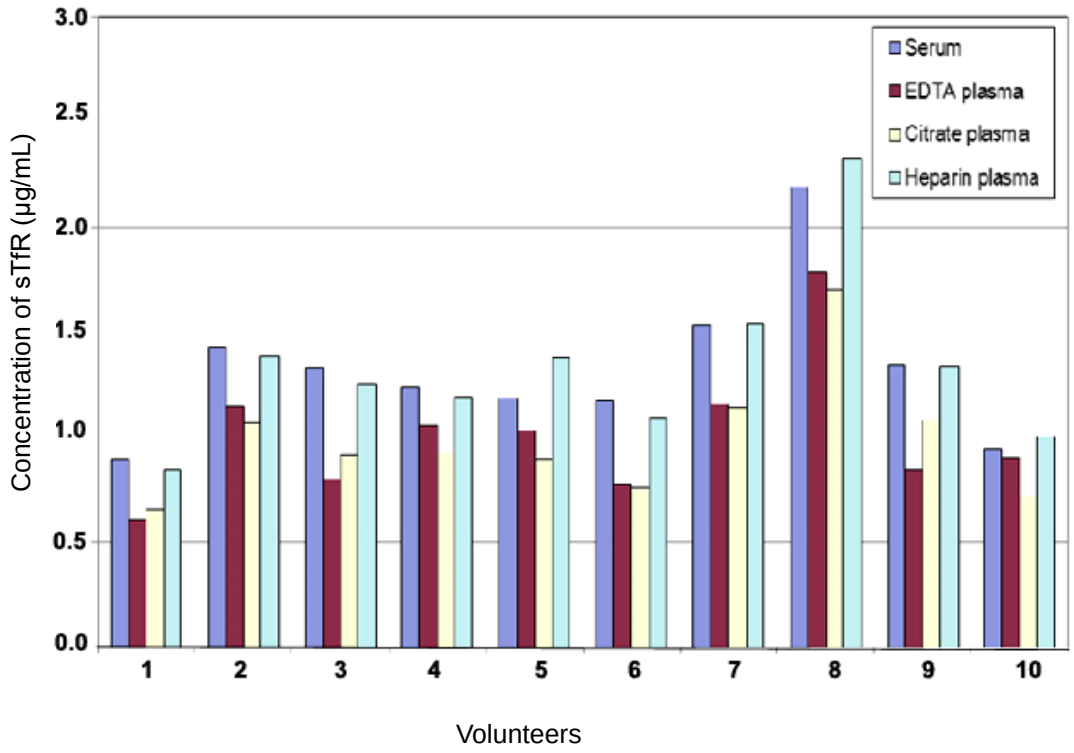


Figure 2: sTfR levels measured in serum and EDTA, citrate and heparin plasma, respectively using TFRC (Human) ELISA Kit.

- 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

- Reference range

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

BMI (kg/m^2)	N	Mean (µg/mL)	Median (µg/mL)	Max. (µg/mL)	Min. (µg/mL)	SD (µg/mL)	SEM (µg/mL)	2.5^{th} - 97.5^{th} 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 abnormal reference ranges for sTfR levels with the assay. Each laboratory should establish a quality control program to monitor the quality of the assay.

- Method comparison

  The TFRC (Human) ELISA Kit was compared to a commercial Immunoturbidimetry (IT).

  The following correlation graph was obtained.

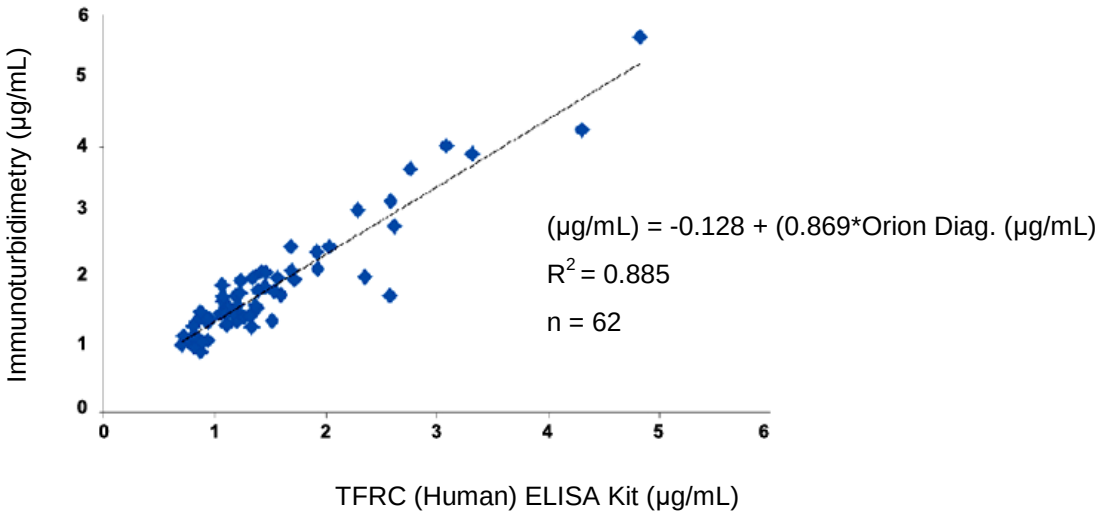


Figure 3: Correlation of TFRC (Human) ELISA Kit results vs. a commercial IT assay.

Resources

Troubleshooting

- 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

- High coefficient of variation (CV)

  Possible explanation:

✓ Improper or inadequate washing

✓ Improper mixing Standards, Quality Controls or samples

References

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Plate Layout

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