BioVendor Group

ENG

Instructions for Use:

HUMAN sTfR ELISA

Catalogue number:

RD 194011100

European Union:

IVD CE

Rest of the world:

For research use only.

BioVendor R&D

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CONTENT

HISTORY OF CHANGES

Previous version	Current version
ENG.008.A	ENG.009.A
A symbol indicating the manufacturer added.	
Chapter 19 “Additional information” added.	

1. INTENDED USE

The RD194011100 Human sTfR ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human soluble transferrin receptor.

Features

- **European Union: for in vitro diagnostic use**
- **Rest of the world: for research use only!**
- The total assay time is less than 3 hours
- The kit measures total soluble transferrin 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

2. 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.

3. INTRODUCTION

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

4. TEST PRINCIPLE

In the BioVendor 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.

5. 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

6. 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 consumable materials and unused contents in accordance with applicable national regulatory requirements

7. REAGENT SUPPLIED

Kit Components	State	Quantity
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

8. MATERIAL REQUIRED BUT NOT SUPPLIED

- Deionized (distilled) wat
- Test tubes for diluting samples
- Glassware (graduated cylinder and bottle) for Wash Solution
- Precise 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)

9. 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.

Centrifuge liquid containing microtube vials before opening.

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 concentrations in serum or another body fluid. Quality Controls serve just for control that the kit works in accordance with IFU and 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.

10. 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.

See Chapter 13 for effect of sample matrix (serum/plasma) on the concentration of sTfR.

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

11. 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 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!
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.

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.

	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.

12. 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: 0.4 µg/ml (from standard curve) x 5 (dilution factor) = 2.0 µg/ml (real concentration in sample).

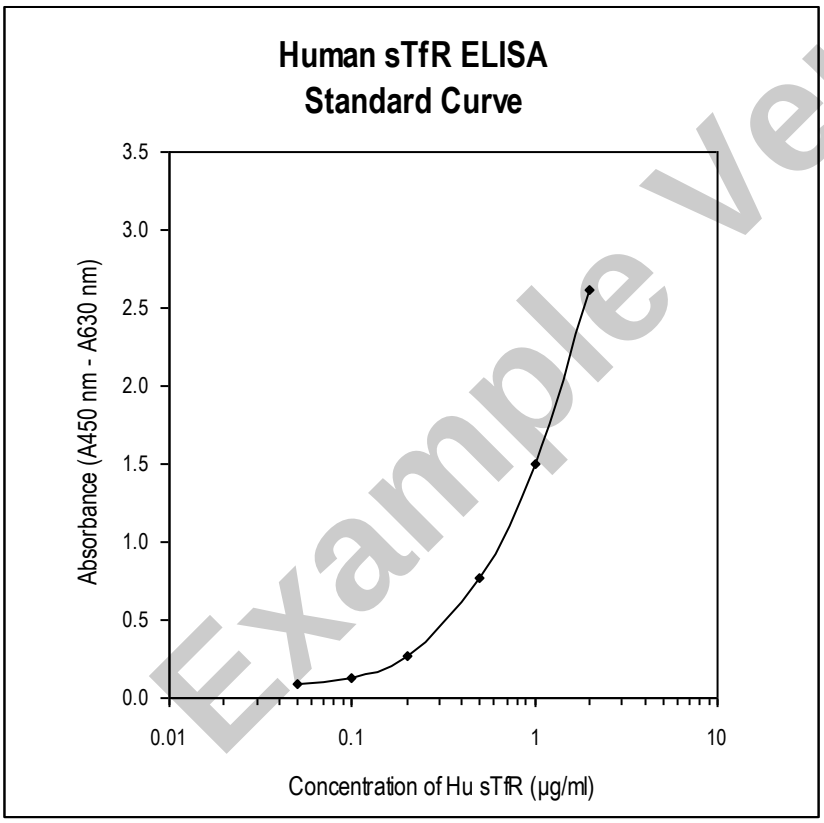


Figure 2: Typical Standard Curve for Human sTfR ELISA.

13. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Human sTfR ELISA are presented in this chapter.

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.
For details please contact us at info@biovendor.com.

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

Presented results are multiplied by respective dilution factor

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 the corresponding 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**

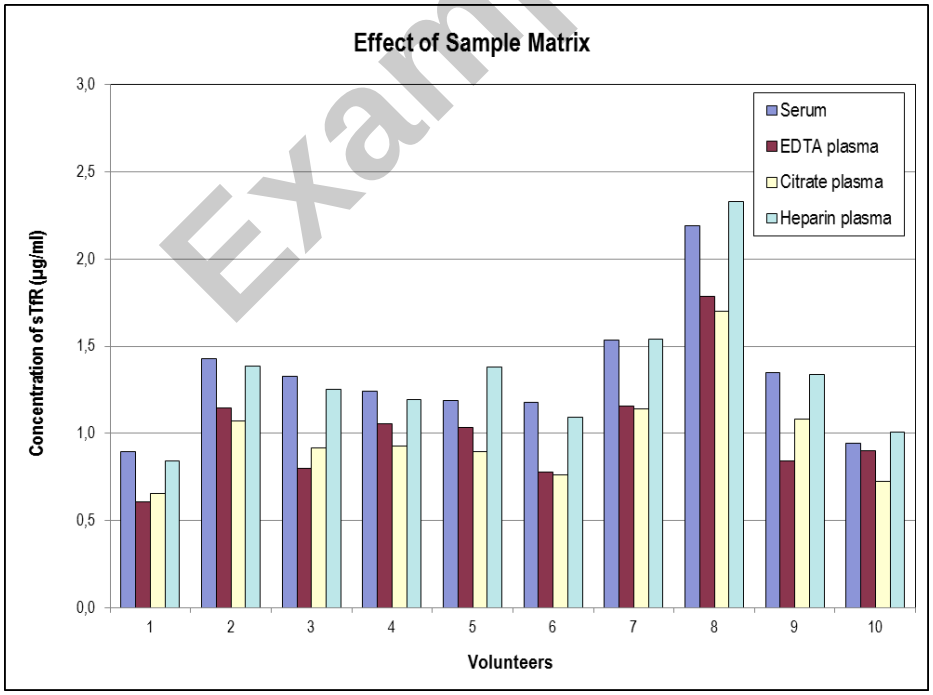


Figure 3: sTfR levels measured in serum and EDTA, citrate and heparin plasma, respectively, using sTfR ELISA

14. 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

15. 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 BioVendor Human sTfR ELISA 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 pathological ranges for sTfR levels with the assay. Each laboratory should establish a quality control program to monitor the quality of the assay.

16. METHOD COMPARISON

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

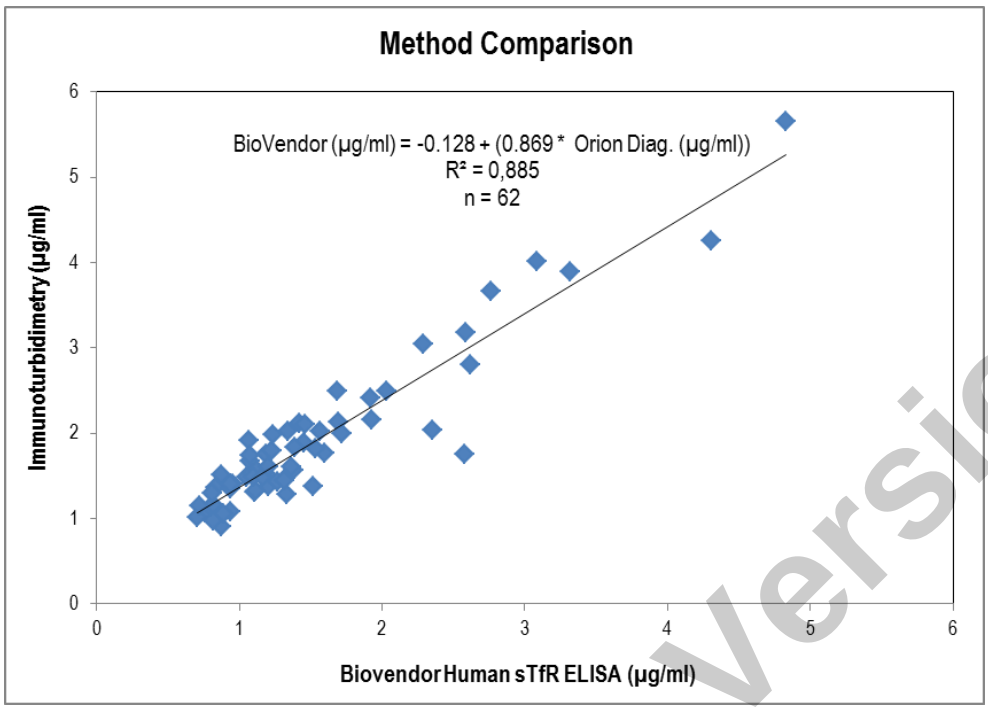


Figure 4: Correlation of BioVendor ELISA results vs. a commercial IT assay.

17. 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

High coefficient of variation (CV)
Possible explanation:
– Improper or inadequate washing
– Improper mixing of Standards, Quality Controls or samples

18. REFERENCES

References to sTfR:

- Lee E. J. et al. Soluble transferrin receptor (sTfR), ferritin, and sTfR/log ferritin index in anemic patients with nonhematologic malignancy and chronic iInflammation. Clinical Chemistry 48, 1118-1121 (2002)
- Raya G. et al.: Soluble transferrin receptor (sTfR): biological variations and reference limits. Clinical Chemistry and Laboratory Medicine, 39, 1162-1168 (2001)
- Cotton F. et al.: Measurement of soluble transferrin receptor by immunoturbidimety and immunonefelometry. Clinical Biochemistry, 33, 263-267 (2000)
- Cook J. D.: The measurement of serum transferrin receptor. The American Journal of the Medical Sciences 318, 269-276 (1999)
- Olivares M. et al.: Usefulness of serum transferrin receptor and serum ferritin in diagnosis of iron deficiency in infancy. American Journal of Clinical Nutrition 72, 1191-1195 (2000)
- Suominen P. et al.: Single values of serum transferrin receptor and transferrin receptor ferritin index can be used to detect true and functional iron deficiency in rheumatoid arthritis patients with anemia. Arthritis & Rheumatism 43, 1016-1020 (2000)
- De Block C. E. M. et al.: Soluble transferrin receptor level. A new marker for iron deficiency anemia, a common manifestation of gastric autoimmunity in type 1 diabetes. Diabetes Care 23, 1384-1388 (2000)
- Kolbe-Busch S. et al. Determination of the soluble transferrin receptor in Serum: Evaluation of two enzyme immunoassays and a particle-enhanced immunonephelometric assay. Clinical Laborators 45, 295-304 (1999)
- Hikawa A. et al.: Soluble transferrin receptor-transferrin complex in serum: measurement by latex agglutination nephelometric asssay. Clinica Chimica Acta 254, 159-172 (1996)
- Flowers C. H. et al.: The clinical measurement of serum transferrin receptor. Journal of Laboratory and Clinical Medicine 114, 368-377 (1989)

References to this product:

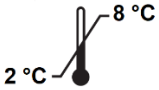
- Fernández-Real J. M. et al. Circulating soluble transferrin receptor according to glucose tolerance status and insulin sensitivity. Diabetes Care 30, 604-608 (2007)

For more references on this product see our web pages at www.biovendor.com

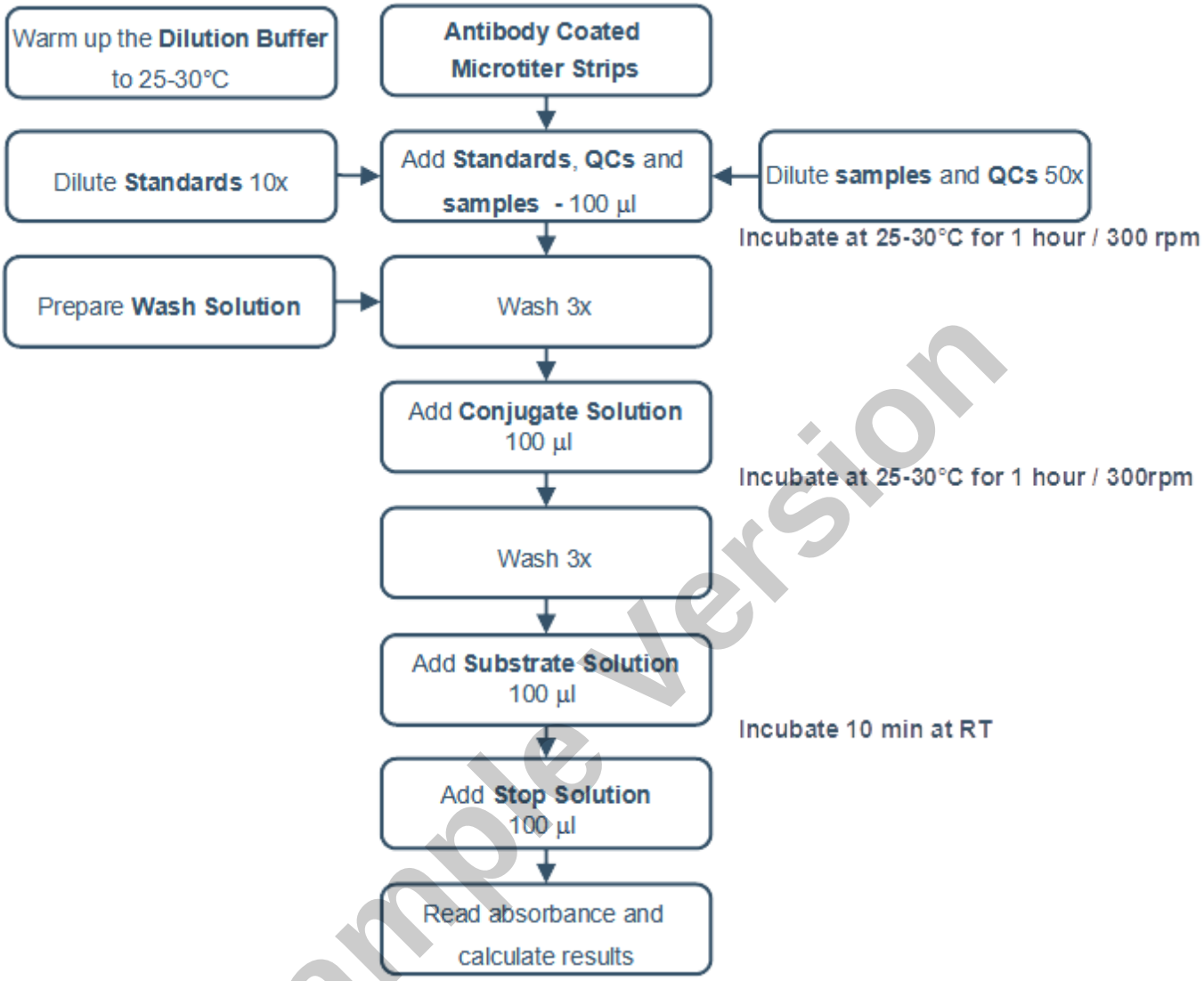
19. ADDITIONAL INFORMATION

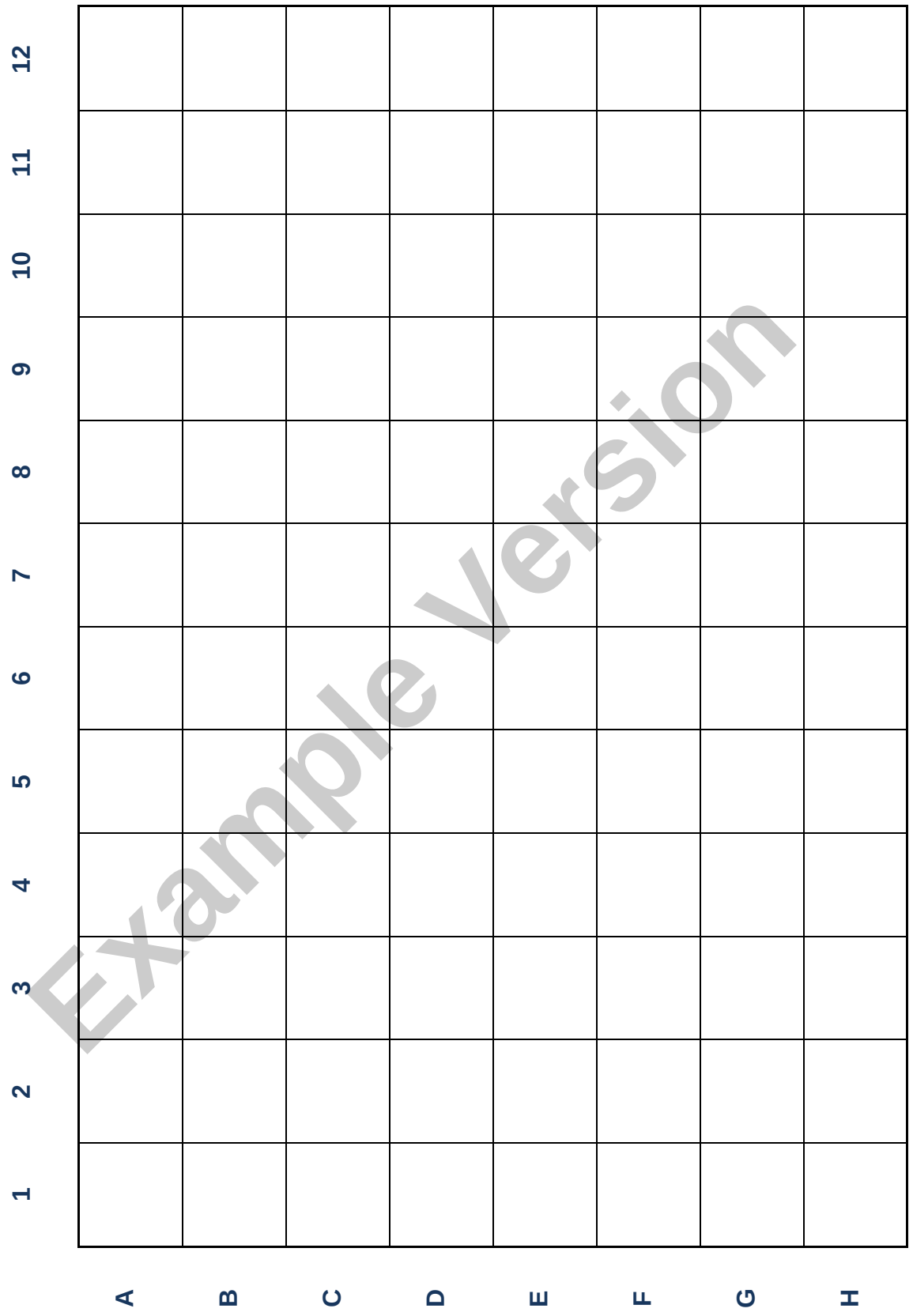
Any serious incident occurring in connection with the device must be reported to the manufacturer and to the competent authority of the Member State in which the user or patient is located.

20. EXPLANATION OF SYMBOLS

	Catalogue number
	Batch code
	Caution
	Use by date
	Temperature limit
	Manufacturer
	Read electronic instructions for use - eIFU
	The content is sufficient for 96 tests
	Biological risks
	In vitro diagnostic medical device
	CE marking of conformity

21. ASSAY PROCEDURE - SUMMARY





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