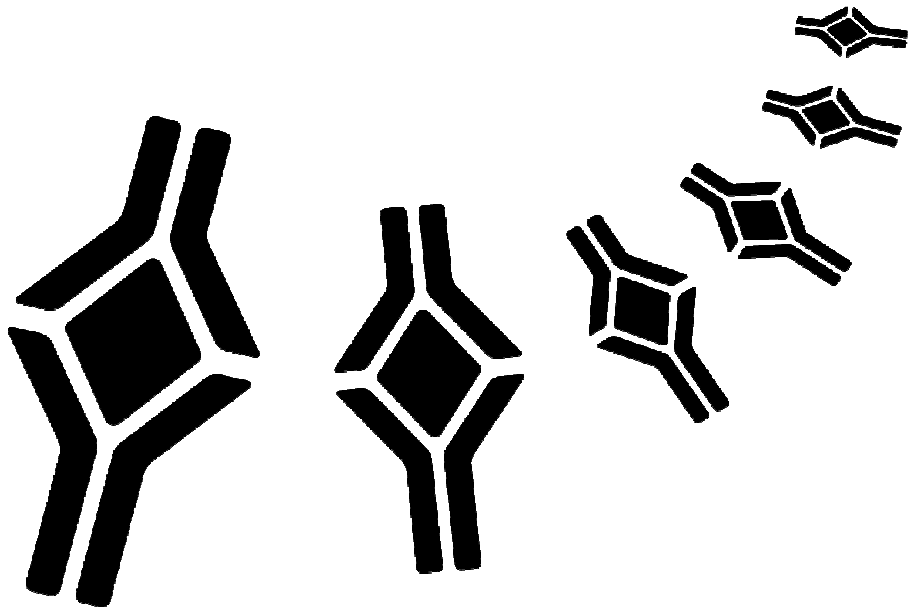
BioVendor
Research
and Diagnostic Products



HUMAN TREFOIL FACTOR 1 ELISA

Product Data Sheet

Cat. No.: RD191158100R

For Research Use Only

VERSION 21 200710

CONTENTS

» **This kit is manufactured by:**
BioVendor – Laboratorní medicína a.s.

» **Use only the current version of Product Data Sheet enclosed with the kit!**

1. INTENDED USE

The RD191158100R Human Trefoil Factor 1 ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human Trefoil Factor 1.

Features

- **For research use only!**
- The total assay time is less than 3 hours
- The kit measures total Trefoil Factor 1 in serum, plasma (EDTA, citrate, heparin) and bronchoalveolar lavage fluid
- Assay format is 96 wells
- Quality Controls are human serum based
- Standard is recombinant protein
- Components of the kit are provided ready to use or lyophilized

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

Trefoil factor 1 (TFF1, pS2) is a small secreted protein with molecular weight of 6.5 kDa (monomers, 14 kDa – dimers). It belongs to the TFF protein family that is characterized by a clover leaf–like disulphide structure named the TFF domain, which is created by 6 cysteines forming three intramolecular bonds. TFF1 contains one trefoil domain, but has a seventh cysteine in position 57 that is essential for formation of dimers. TFF1 exist as both monomers and dimers (homo- and heterodimers – with gastrokine 2).
The most abundant expression of TFF1 is found in the GI tract (especially in stomach, colon and pancreas) where it is co-localised with mucins, usually with MUC5AC. It is probable that TFF1 is closely connected with healing and stabilisation of the mucin layer. TFF1 was found in significant amounts in ulcer associated cell lineage UACL, where EGF (epidermal growth factor) is also present. The hypothesis that TFF1 expression is influenced by EGF has been proposed, and this has been supported by a study on EGF KO mice which had lower levels of TFF1.
A study examining people with Crohn´s disease and inflammatory bowel disease showed that TFF1 level in serum is increased during the inflammatory state. TFF1 is also highly expressed in the trachea and its level increases after administration of allergen, indicating thatTFF1 could be associated with asthma. Another study found that TFF1 levels are high in septic patients and that the level correlates with prognosis of the septic state.
High levels of TFF1 in serum were also found in patients with prostate and other types of cancer (breast, colon and ovarian tumors) but its prognostic value has not yet been proved. The exact function of TFF1 is not yet fully understood.

<u>Areas of investigation:</u>
Immune Response, Infection and Inflammation
Energy metabolism and body weight regulation
Oncology

4. TEST PRINCIPLE

In the BioVendor Human Trefoil Factor 1 ELISA, standards, quality controls and samples are incubated in microtitrate plate wells pre-coated with polyclonal anti-human Trefoil Factor 1 antibody. After 60 minutes incubation and washing, polyclonal anti-human Trefoil Factor 1 antibody, conjugated with horseradish peroxidase (HRP) is added to the wells and incubated for 60 minutes with captured Trefoil Factor 1. 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 Trefoil Factor 1. A standard curve is constructed by plotting absorbance values against concentrations of Trefoil Factor 1 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. However, these materials should be handled as potentially infectious, as no test can guarantee the complete absence of infectious agents
- This kit contains components of animal origin. These materials should be handled as potentially infectious
- 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 of 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
Master Standard	lyophilized	1 vial
Quality Control HIGH	lyophilized	2 vials
Quality Control LOW	lyophilized	2 vials
Dilution Buffer	ready to use	20 ml
Wash Solution Conc. (10x)	concentrate	100 ml
Substrate Solution	ready to use	13 ml
Stop Solution	ready to use	13 ml
Product Data Sheet + Certificate of Analysis	-	1 pc

8. 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 10-1000 µl with disposable tips
- Multichannel pipette to deliver 100 µl with disposable tips
- Absorbent material (e.g. paper towels) for blotting the microtitrate 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)

9. PREPARATION OF REAGENTS

» **All reagents need to be brought to room temperature 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 or lyophilized:**

Human Trefoil Factor 1 Master Standard
Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution of standard!!!
Reconstitute the lyophilized Master Standard with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam). The resulting concentration of TFF1 in the stock solution is **4 ng/ml**.

Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	*Dilution Buffer*	*Concentration*
Stock	-	4 ng/ml
250 µl of std. 4 ng/ml	250µl	2 ng/ml
250 µl of std. 2 ng/ml	250µl	1 ng/ml
250 µl of std. 1 ng/ml	250µl	0.5 ng/ml
250 µl of std. 0.5 ng/ml	250µl	0.25 ng/ml
250 µl of std. 0.25 ng/ml	250µl	0.125 ng/ml

Prepared Standards are ready to use, do not dilute them.
Stability and storage:
Standard stock solution (4 ng/ml) must be used immediately or aliquoted and frozen at –20°C for 3 months. Avoid repeated freeze/thaw cycles.
Do not store the diluted standard solutions.

Quality Controls HIGH, LOW
Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution and for current Quality Controls concentrations!!!
Reconstitute each Quality Control (HIGH and LOW) with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam).
The reconstituted Quality Controls are ready to use, do not dilute them.

Stability and storage:
The reconstituted Quality Controls must be used immediately. Avoid repeated freeze/thaw cycles.
Do not store the reconstituted Quality Controls.

Wash Solution Conc. (10x)
Dilute Wash Solution Concentrate (10x) ten-fold in 900 ml of distilled water to prepare a 1x working solution, e.g. 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 Trefoil Factor 1 in serum, plasma (EDTA, citrate, heparin) and bronchoalveolar lavage fluid.

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 serum, plasma or BALF samples 5x with Dilution Buffer just prior to the assay (e.g**. 30** µl of sample + 120 µl of Dilution Buffer when assaying samples as singlets or preferably **60** µl of sample + 240 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 stability of serum and plasma samples when stored at 2-8°C, effect of freezing/thawing and effect of sample matrix (serum/plasma) on the concentration of Trefoil Factor 1.

Note: It is recommended to use a precision 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 room temperature (ca. 25°C) for **60 minutes**, shaking at ca. 300 rpm on an orbital microplate shaker.
3. Wash the wells 5-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 room temperature (ca. 25°C) for **60 minutes**, shaking at ca. 300 rpm on an orbital microplate shaker. Incubation without shaking is the alternative that requires to extent incubation with substrate – see paragraph 8.
6. Wash the wells 5-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. The incubation time may be extended [up to 20 minutes] if the reaction temperature is below than 20°C. Do not shake with 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 wavelength 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: 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 Trefoil Factor 1 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 4**	**Blank**	Sample 8	Sample 16	Sample 24	Sample 32
B	**Standard 2**	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
C	**Standard 1**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
D	**Standard 0.5**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
E	**Standard 0.25**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
F	**Standard 0.125**	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 Trefoil Factor 1 (ng/ml) in samples.

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

The measured concentration of samples calculated from the standard curve must be multiplied by their respective dilution factor, because samples have been diluted prior to the assay; e.g. 0.3 ng/ml (from standard curve) x 5 (dilution factor) = 1.5 ng/ml.

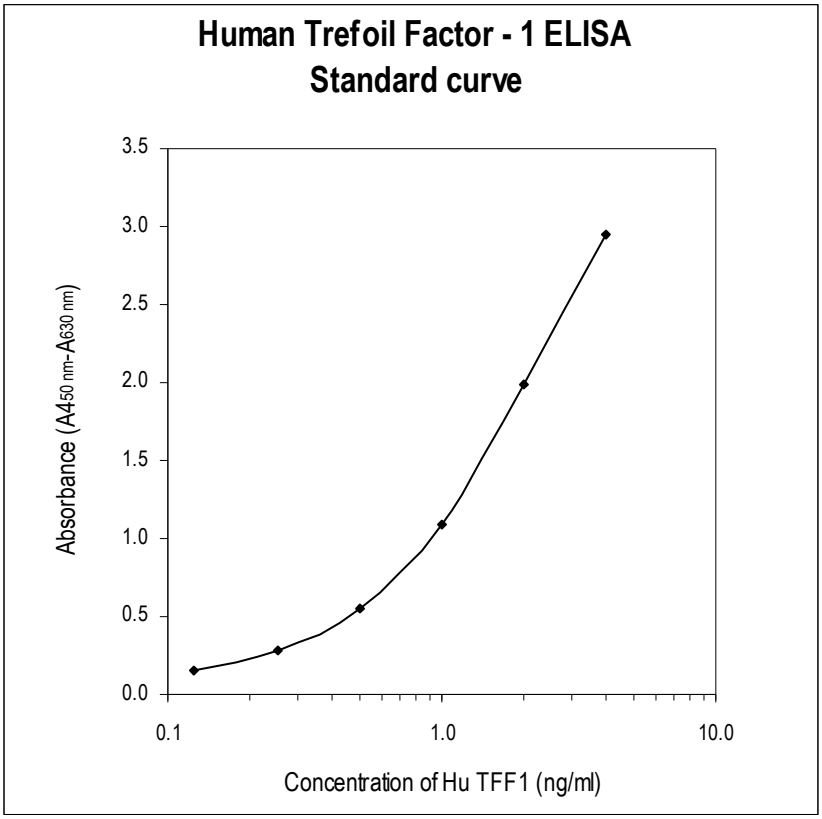


Figure 2: Typical Standard Curve for Human Trefoil Factor 1 ELISA.

13. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Human Trefoil Factor 1 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 Trefoil Factor 1 values in wells and is 0.019 ng/ml.
*Dilution Buffer is pipetted into blank wells.

- **Limit of assay**

Results exceeding Trefoil Factor 1 level of 4 ng/ml should be repeated with more diluted samples. Dilution factor needs to be taken into consideration in calculating the Trefoil Factor 1 concentration.

- **Specificity**

The antibodies used in this ELISA are specific for human Trefoil Factor 1.

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	no
Hamster	no
Horse	no
Monkey	no
Mouse	no
Pig	no
Rabbit	no
Rat	no
Sheep	no

» **Presented results are multiplied by respective dilution factor**

- **Precision**

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

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	7.88	0.11	7.31
2	13.47	0.21	8.21

Inter-assay (Run-to-Run) (n=6)

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	2.03	0.08	3.95
2	7.89	0.33	4.22

- **Spiking Recovery**

Serum samples were spiked with different amounts of human Trefoil Factor 1, diluted with Dilution Buffer 400x and assayed.

Sample	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
1	1.13	-	-
	1.88	2.13	88.0
	3.13	3.13	100.1
	5.32	5.13	103.7
2	7.00	-	-
	8.69	8.00	108.6
	9.54	9.00	106.0
	12.42	11.00	112.9

- **Linearity**

Serum samples were serially diluted with Dilution Buffer after primary dilution 5x and assayed.

Sample	*Dilution*	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
1	-	18.389	-	-
	2x	9.664	9.19	105.1
	4x	4.575	4.60	99.5
	8x	2.139	2.30	93.1
2	-	19.194	-	-
	2x	10.053	9.60	104.8
	4x	4.939	4.80	102.9
	8x	2.271	2.40	94.7

- **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 (ng/ml)*	*Plasma (ng/ml)*		
		EDTA	*Citrate*	*Heparin*
1	0.648	0.436	0.861	0.371
2	0.350	0.351	0.371	0.285
3	0.770	0.829	0.874	0.709
4	4.251	3.243	4.568	3.933
5	4.049	2.428	3.417	2.770
6	0.902	1.041	1.386	0.793
7	1.814	1.444	1.886	1.389
8	1.327	2.318	1.928	1.518
9	1.039	1.537	1.471	1.029
10	0.780	1.154	1.107	0.798
Mean (ng/ml)	**1.593**	**1.478**	**1.787**	**1.360**
Mean Plasma/Serum (%)	**-**	**94%**	**112%**	**88%**
Coefficient of determination R^2	**-**	**0.76**	**0.94**	**0.94**

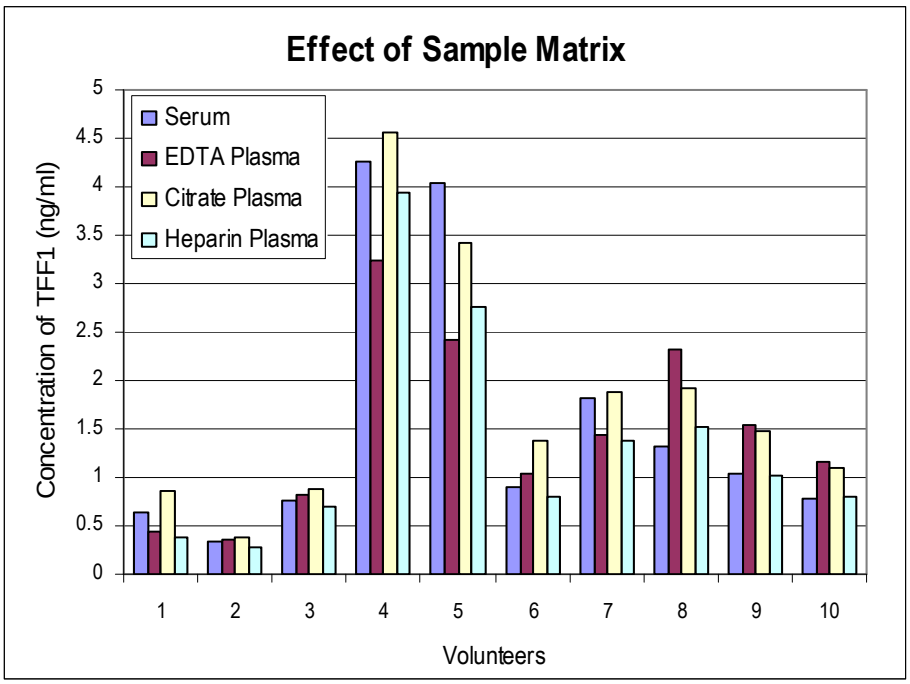


Figure 3: Trefoil Factor 1 levels measured using Human Trefoil Factor 1 ELISA from 10 individuals using serum, EDTA, citrate and heparin plasma, respectively.

- **Stability of samples stored at 2-8°C**

Samples should be stored at -20°C. However, no decline in concentration of Trefoil Factor 1 was observed in serum and plasma samples after 14 days when stored at 2-8°C. To avoid microbial contamination, samples were treated with ε-aminocaproic acid and sodium azide, resulting in the final concentration of 0.03% and 0.1%, respectively.

Sample	*Incubation Temp, Period*	*Serum (ng/ml)*	*Plasma (ng/ml)*		
			EDTA	*Citrate*	*Heparin*
1	-20°C	0.7	0.9	0.8	0.6
	2-8°C, 1 day	0.7	0.9	0.8	0.5
	2-8°C, 7 days	0.8	0.9	0.8	0.5
2	-20°C	3.9	3.0	4.1	3.7
	2-8°C, 1 day	4.1	3.4	4.0	3.4
	2-8°C, 7 days	4.3	3.8	4.1	3.5
3	-20°C	3.2	2.7	3.3	2.6
	2-8°C, 1 day	3.4	2.7	3.3	2.4
	2-8°C, 7 days	3.5	2.9	3.2	2.5

- **Effect of Freezing/Thawing**

No decline was observed in concentration of human Trefoil Factor 1 in serum and plasma samples after repeated (5x) freeze/thaw cycles. However it is recommended to avoid unnecessary repeated freezing/thawing of the samples.

Sample	*Number of f/t cycles*	*Serum (ng/ml)*	*Plasma (ng/ml)*		
			EDTA	*Citrate*	*Heparin*
1	1x	1.2	1.4	1.4	1.2
	3x	1.2	1.4	1.5	1.2
	5x	1.3	1.4	1.5	1.3
2	1x	0.7	1.3	0.9	0.9
	3x	0.7	1.3	1.0	0.9
	5x	0.7	1.3	0.8	0.9
3	1x	0.5	0.6	0.5	0.6
	3x	0.4	0.6	0.5	0.6
	5x	0.4	0.6	1.5	0.6

14. DEFINITION OF THE STANDARD

The recombinant human Trefoil Factor 1 is used as the Standard. The recombinant human TFF1, produced in *E. coli*, is 7.9 kDa protein containing 70 amino acid residues of the human TFF1 and 10 additional amino acid residues- His Tag.

15. PRELIMINARY POPULATION AND CLINICAL DATA

The following results were obtained when serum samples from 61 unselected donors (33 men + 27 women) 23 - 65 years old were assayed with the Biovendor Human Trefoil Factor 1 ELISA in our laboratory:

- **Age and Sex dependent distribution of Trefoil Factor 1**

Sex	Age (years)	n	Mean	SD	Min	Max
			Trefoil Factor 1 (ng/ml)			
Men	20-39	15	0.64	0.91	0	2.84
	40-69	18	0.8	1.15	0	4.84
Women	20-39	16	0.49	0.91	0	3.6
	40-69	12	0.36	0.54	0	1.5

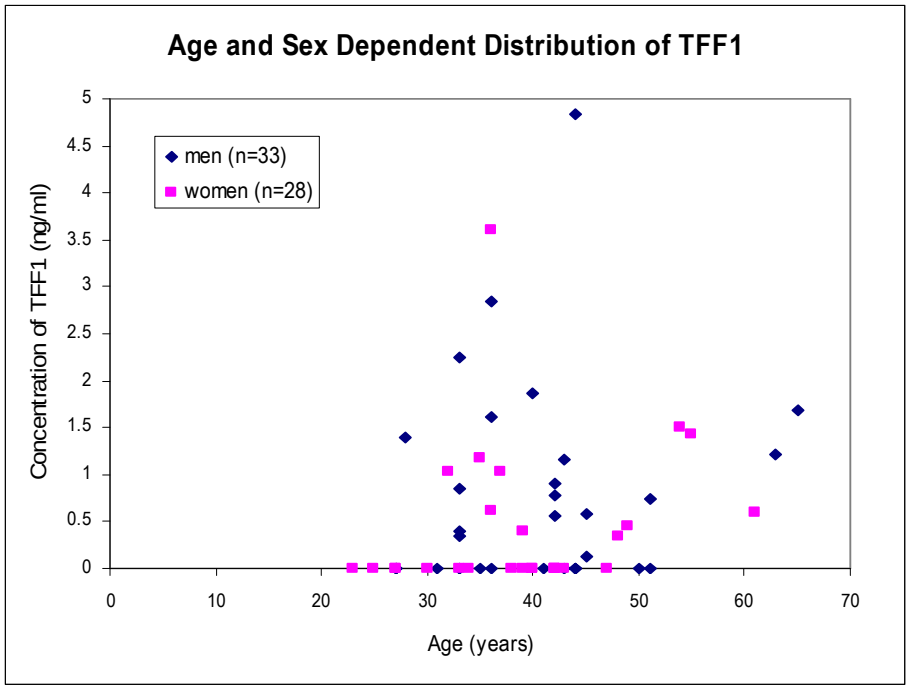


Figure 4: Human Trefoil Factor 1 concentration plotted against donor age and sex.

- **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 sample in the assay. Each laboratory should establish its own normal and pathological references ranges for TFF1 levels with the assay.

16. METHOD COMPARISON

The BioVendor Human Trefoil Factor 1 ELISA has not been compared to any commercial immunoassay.

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 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

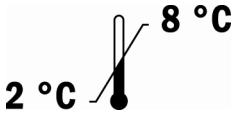
18. REFERENCES

References to Trefoil Factor 1:

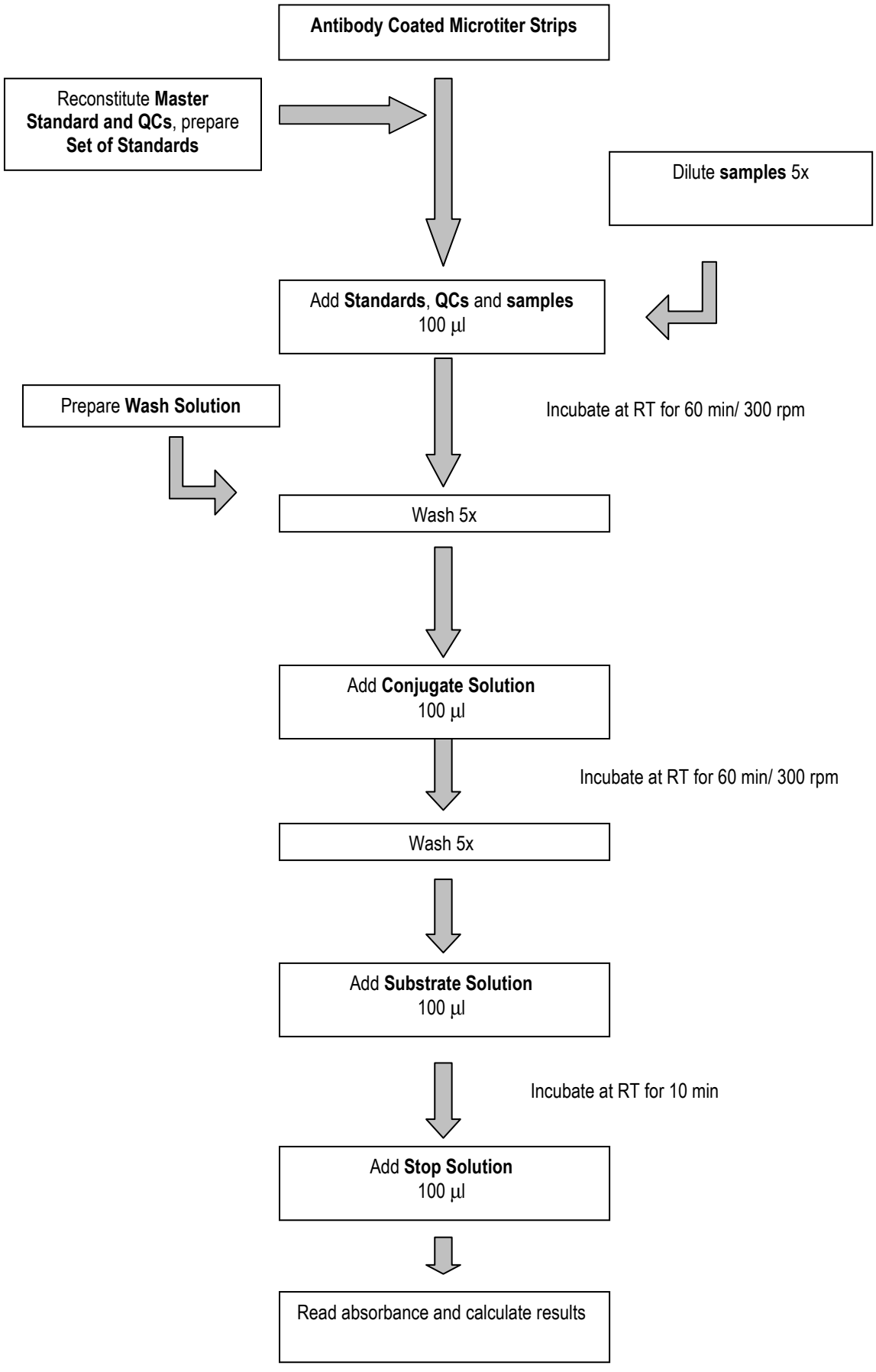
- Kjellev S: The trefoil factor family – small peptides with multiple functionalities. Cell Mol Life Sci 2009; 66: 1350 – 1369
- Vestergaard EM, Brynskov J, Ejskjær K, Clausen JT, Thim L, Nexø E, Poulsen SS: Immunoassays of human trefoil factors 1 and 2: measured on serum from patients with inflammatory disease. Scan J Lab Invest. 2004; 64: 146 – 156
- Oertel M, Graness A., Thim L., Bühling F., Kalbacher H., Hoffmann W.: Trefoil Factor Family – pepdtides promote migration of human bronchial epithelial cells. Am J Respir Cell Mol Biol 2001; 25: 418 – 424
- Kouznetsova I., Chwieralski C. E., Bälder R., Hinz M., Braun A., Krug N., Hoffmann W.: Induced trefoil factor family 1 expression by trans – differentiating clara cells a murine asthma model. Am J Resp Cell Mol Biol 2007; 36: 286 – 295
- Madsen J., Nielsen O., Tornøe I., Thim L., Holmskov U.: Tissue localization of human trefoil factors 1, 2 and 3. JHC 2007; 55: 505 – 513
- Vestergaard E.M., Borne M., Poulsen S.S., Nexø E., Tørring N.: Plasma levels of trefoil factor are increased in patients with advanced prostate cancer. Clin Canc Res 2006; 12: 807 – 812
- Hesselund SM, Vestergaard EM, Milman N, Poulsen SS, Nexø E: Circulating serum trefoil factors increase dramatically during pregnancy. Scand J Lab Invest 2008 68 (5): 369 - 374

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

19. EXPLANATION OF SYMBOLS

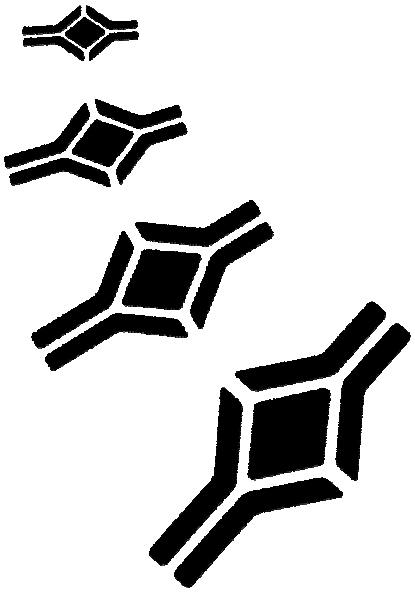
REF	Catalogue number
Cont.	Content
LOT	Lot number
	See instructions for use
	Biological hazard
	Expiry date
	Storage conditions
	Identification of packaging materials

Assay Procedure Summary



	A	B	C	D	E	F	G	H
12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								

BioVendor
Research
and Diagnostic Products



HEADQUARTERS: **BioVendor - Laboratorní medicína a.s.**	**CTPark Modrice Evropska 873**	**664 42 Modrice CZECH REPUBLIC**	**Phone: +420-549-124-185 Fax: +420-549-211-460**	**E-mail: info@biovendor.com Web: www.biovendor.com**
EUROPEAN UNION: BioVendor GmbH	Im Neuenheimer Feld 583	D-69120 Heidelberg GERMANY	Phone: +49-6221-433-9100 Fax: +49-6221-433-9111	E-mail: infoEU@biovendor.com
USA, CANADA AND MEXICO: BioVendor LLC	1463 Sand Hill Road Suite 227	Candler, NC 28715 USA	Phone: +1-828-670-7807 +1-800-404-7807 Fax: +1-828-670-7809	E-mail: infoUSA@biovendor.com
CHINA - Hong Kong Office: BioVendor Laboratories Ltd	Room 4008 Hong Kong Plaza, No.188	Connaught Road West Hong Kong, CHINA	Phone: +852-2803-0523 Fax: +852-2803-0525	E-mail: infoHK@biovendor.com
CHINA – Mainland Office: BioVendor Laboratories Ltd	Room 2405 YiYa Tower TianYu Garden, No.150	Lihe Zhong Road Guang Zhou, CHINA	Phone: +86-20-87063029 Fax: +86-20-87063016	E-mail: infoCN@biovendor.com