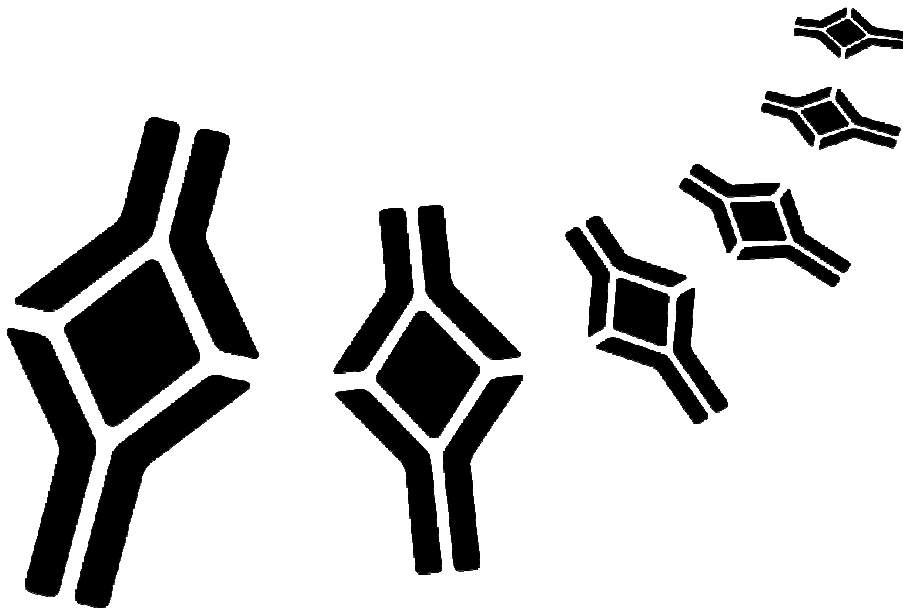


**BioVendor**

Research  
and Diagnostic Products



## HUMAN TREFOIL FACTOR 3 ELISA

Product Data Sheet

Cat. No.: RD191160200R

For Research Use Only

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➤➤ 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

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The RD191160200R Human Trefoil Factor 3 ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human total trefoil factor 3 protein (TFF3).

### »» Features

- **It is intended for research use only**
- The total assay time is less than 3.5 hours
- The kit measures total TFF3 in serum, plasma (EDTA, citrate, heparin), BALF, and urine samples.
- Special Dilution Buffer (Cat. No.: C005114) needed for the dilution of urine and BALF samples is not included and can be obtained from BioVendor. For details please contact us at [info@biovendor.com](mailto:info@biovendor.com)
- Assay format is 96 wells
- Quality Controls are human serum based
- Standard is recombinant protein based
- Components of the kit are provided ready to use, concentrated or lyophilized

## 2. STORAGE, EXPIRATION

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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

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Human trefoil factor 3 (TFF3, also known as intestinal trefoil factor) belongs together with TFF1 and TFF2 to a small group of mucin-associated peptides. TFF3 contains seven cysteine residues, six of which form disulfide bonds to create a characteristic three-leafed structure. Due to its compact structure, TFF3 is extremely resistant toward acids, proteolytical cleavage or heat degradation. Monomeric form of TFF3 consists of 60 amino acids and has 6.7 kDa, while the dimer (13.1 kDa) consists of 118 amino acids.

TFF3 is expressed mainly in gastrointestinal tract, in the mucous cells of the small and large intestine, where it maintains the integrity of mucous layer and in cooperation with mucins protects the gastrointestinal epithelial cells against various injurious agents. However, TFF3 was also detected in salivary glands, posterior pituitary gland and in the inner ear. Secretion of TFF3 is triggered by the presence of certain inflammation mediators and neurotransmitters. Studies showed that oral administration of TFF3 in rats protects gastric mucosa from damage. Over-expression of TFF3 occurs at the sites of damage of the gastrointestinal tract, e.g. peptic ulcer or inflammatory bowel disease. Patients suffering from these diseases have increased levels of TFF3 in serum. TFF3 was reported to be over-expressed also in patients with various neoplasms including intestinal, pancreatic and prostate carcinomas. On the contrary, its expression decreases in thyroid follicular carcinomas. In vitro studies showed that in breast cancer cells, expression of TFF3 is regulated by the level of estrogen.

Recent study with human and rodent pancreatic islet  $\beta$ -cells has demonstrated that TFF3 overexpression increases their proliferation. Both major forms of diabetes involve a decline in islet  $\beta$ -cells mass and their controlled expansion would have great potential utility for treatment of this diseases.

Another study with rats has shown that urinary TFF3 protein levels were markedly reduced in response to renal tubular injury, while his levels did not respond to nonrenal toxicants.

#### Areas of investigation:

Neoplasmas

Carcinomas

Diabetes mellitus

Kidney tubular injury

## 4. TEST PRINCIPLE

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In the BioVendor Human Trefoil Factor 3 ELISA, standards, quality controls and samples are incubated in microplate wells pre-coated with polyclonal anti-human TFF3 antibody. After 60 minutes incubation and washing, biotin labelled polyclonal anti-human TFF3 antibody is added and incubated with captured TFF3 for 60 minutes. After another washing, streptavidin-HRP conjugate is added. After 30 minutes incubation and the last washing step, the remaining 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 TFF3. 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

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- **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
- 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

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- 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

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<i>Kit Components</i>	<i>State</i>	<i>Quantity</i>
Antibody Coated Microtiter Strips	ready to use	96 wells
Biotin Labelled Antibody Conc. (50x)	concentrated	0.28 ml
Streptavidin-HRP Conjugate	ready to use	13 ml
Master Standard	lyophilized	2 vials
Quality Control HIGH	lyophilized	2 vials
Quality Control LOW	lyophilized	2 vials
Biotin-Ab Diluent	ready to use	13 ml
Dilution Buffer	ready to use	20 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

## 8. MATERIAL REQUIRED BUT NOT SUPPLIED

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- Special Dilution Buffer (Cat. No.: C005114) needed for the dilution of urine and BALF samples
- Deionized (distilled) water
- Test tubes for diluting samples
- Glassware (graduated cylinder and bottle) for Wash Solution (Dilution Buffer)
- Precision pipettes to deliver 5-1000  $\mu$ l with disposable tips
- Multichannel pipette to deliver 100  $\mu$ l 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  $450 \pm 10$  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

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- 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 desiccant and seal carefully. Remaining Microtiter Strips are stable 3 months when stored at 2-8°C and protected from the moisture.

### **Streptavidin-HRP Conjugate**

#### **Biotin-Ab Diluent**

#### **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 TFF3 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 the TFF3 in the stock solution is **2.4 ng/ml**.

Prepare set of standards using Dilution Buffer as follows:

<i>Volume of Standard</i>	<i>Dilution Buffer</i>	<i>Concentration</i>
Stock	-	2.4 ng/ml
250 µl of stock	250 µl	1.2 ng/ml
250 µl of 1.2 ng/ml	250 µl	0.6 ng/ml
250 µl of 0.6 ng/ml	250 µl	0.3 ng/ml
250 µl of 0.3 ng/ml	250 µl	0.15 ng/ml
250 µl of 0.15 ng/ml	250 µl	0.075 ng/ml

**Prepared Standards are ready to use, do not dilute them.**

Stability and storage:

**Do not store the Standard stock solutions and set of standards.**

**Quality Controls HIGH, LOW**

**Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution and for current Quality Control concentration!!!**

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).

**Reconstituted Quality Controls are ready to use, do not dilute them.**

Stability and storage:

**Do not store the reconstituted 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 PDS and CoA and that ELISA test was carried out properly.*

**Biotin Labelled Antibody Conc. (50x)**

Prepare the working Biotin Labelled Antibody solution by adding 1 part Biotin Labelled Antibody Conc. (50x) with 49 parts Biotin-Ab Diluent. Example: 20 µl of Biotin Labelled Antibody Conc. (50x) + 980 µl of Biotin-Ab Diluent for 1 strip (8 wells).



#### Stability and storage:

Opened Biotin Labelled Antibody Conc. (50x) is stable 3 months when stored at 2-8°C. **Do not store the diluted Biotin Labelled Antibody solution.**

#### **Wash Solution Conc. (10x)**

Dilute Wash Solution Conc. (10x) ten-fold in distilled water to prepare a 1x working solution. Example: 100 ml of Wash Solution Conc. (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 Conc. (10x) is stable 3 months when stored at 2-8°C.

## 10. PREPARATION OF SAMPLES

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The kit measures human TFF3 in serum, plasma (EDTA, citrate, heparin), BALF and urine samples.

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.

#### **Serum and plasma samples:**

Dilute samples 5x with Dilution Buffer just prior to the assay, e.g. 30 µl of sample + 120 µl of Dilution Buffer for singlets and 50 µl of sample + 200 µl of Dilution Buffer for duplicates. **Mix well** (not to foam). Vortex is recommended.

#### **Urine samples:**

Special Dilution Buffer (Cat. No.: C005114, not included in the kit) is needed for the dilution of urine samples. Dilute urine samples 20x with the special Dilution Buffer just prior to the assay, e.g. 10 µl of sample + 190 µl of Dilution Buffer for singlets and 15 µl of sample + 285 µl of Dilution Buffer for duplicates. **Mix well** (not to foam). Vortex is recommended.

Results exceeding urine TFF3 level of 2 ng/ml should be repeated with more dilute samples. It is recommended to dilute urine samples in next assay 100x with the special Dilution Buffer just prior to the assay, e.g. 5 µl of sample + 495 µl of Dilution Buffer for singlets and duplicates.

**Mix well** (not to foam). Vortex is recommended.

Dilution factor have to be taken into consideration in calculating the TFF3 concentration.

#### **BALF samples:**

Special Dilution Buffer (Cat. No.: C005114, not included in the kit) is needed for the dilution of BALF samples. Dilute BALF samples 5x with the special Dilution Buffer just prior to the assay,

e.g. 30 µl of sample + 120 µl of Dilution Buffer for singlets and 50 µl of sample + 200 µl of Dilution Buffer for duplicates. **Mix well** (not to foam). Vortex is recommended.

Results exceeding BALF TFF3 level of 2 ng/ml should be repeated with more dilute samples. It is recommended to dilute BALF samples in next assay 50x with the special Dilution Buffer just prior to the assay, e.g. 5 µl of sample + 245 µl of Dilution Buffer for singlets and duplicates.

**Mix well** (not to foam). Vortex is recommended.

Dilution factor have to be taken into consideration in calculating the TFF3 concentration.

Stability and storage:

Serum samples should be stored at -20°C, or preferably at -70°C for long-term storage. Urine and BALF samples should be stored at -70°C. 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 human TFF3.

*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

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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 **1 hour**, shaking at ca. 300 rpm on an orbital microplate shaker.
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 Biotin Labelled Antibody solution into each well.
5. Incubate the plate at room temperature (ca. 25°C) for **1 hour**, shaking at ca. 300 rpm on an orbital microplate shaker.
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 Streptavidin-HRP Conjugate into each well.
8. Incubate the plate at room temperature (ca. 25°C) for **30 min**, shaking at ca. 300 rpm on an orbital microplate shaker.
9. 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.
10. 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.
11. 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 20°C. Do not shake the plate during the incubation.
12. Stop the colour development by adding **100 µl** of Stop Solution.
13. Determine the absorbance of each well on 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 12.**

*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 TFF3 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.*

	<b>strip 1+2</b>	<b>strip 3+4</b>	<b>strip 5+6</b>	<b>strip 7+8</b>	<b>strip 9+10</b>	<b>strip 11+12</b>
<b>A</b>	<b>Standard 2.4</b>	<b>Blank</b>	Sample 8	Sample 16	Sample 24	Sample 32
<b>B</b>	<b>Standard 1.2</b>	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
<b>C</b>	<b>Standard 0.6</b>	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
<b>D</b>	<b>Standard 0.3</b>	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
<b>E</b>	<b>Standard 0.15</b>	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
<b>F</b>	<b>Standard 0.075</b>	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
<b>G</b>	<b>QC HIGH</b>	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
<b>H</b>	<b>QC LOW</b>	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 TFF3 ng/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 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. 0.5 ng/ml (from standard curve) x 5 (dilution factor) = 2.5 ng/ml.**

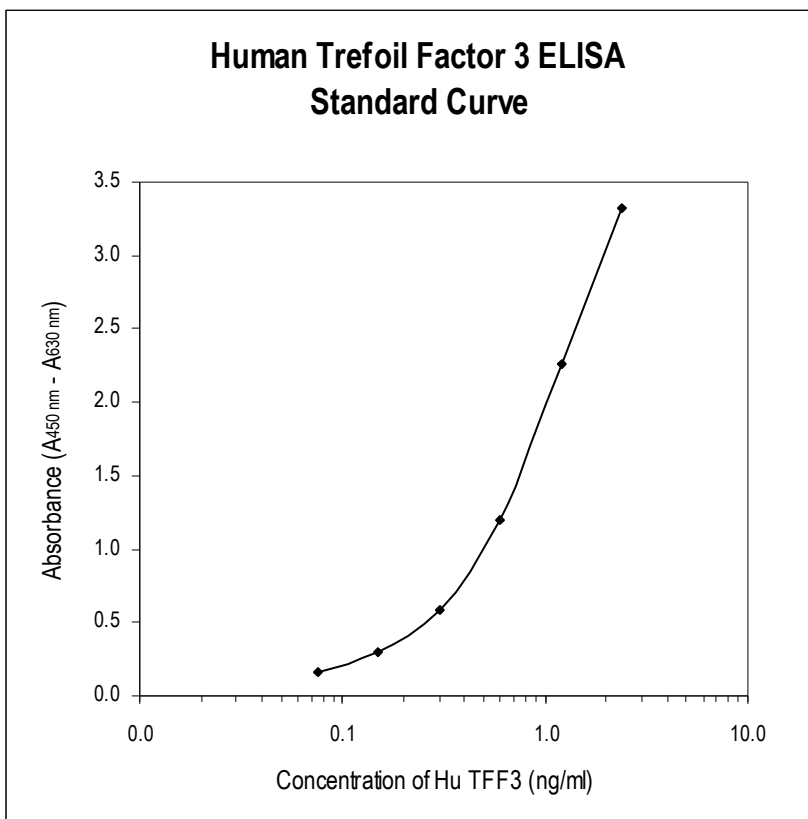


Figure 2: Typical Standard Curve for Human Trefoil Factor 3 ELISA.

## 13. PERFORMANCE CHARACTERISTICS

➤➤ Typical analytical data of BioVendor Human Trefoil Factor 3 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:  $A_{\text{blank}} + 3 \times \text{SD}_{\text{blank}}$ ) is calculated from the real human TFF3 values in wells and is 0.007 ng/ml.

\*Dilution Buffer is pipetted into blank wells.

- **Limit of assay**

Results exceeding TFF3 level of 2.4 ng/ml should be repeated with more diluted samples. Dilution factor needs to be taken into consideration in calculating the TFF3 concentration.

- **Specificity**

The antibodies used in this ELISA are specific for human TFF3.

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

For details please contact us at [info@biovendor.com](mailto:info@biovendor.com).

<i>Mammalian serum sample</i>	<i>Observed crossreactivity</i>
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)

<i>Sample</i>	<i>Mean (ng/ml)</i>	<i>SD (ng/ml)</i>	<i>CV (%)</i>
1	1.18	0.07	5.6
2	7.11	0.54	7.6

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

<i>Sample</i>	<i>Mean (ng/ml)</i>	<i>SD (ng/ml)</i>	<i>CV (%)</i>
1	1.57	0.10	6.4
2	8.77	0.66	7.5

• **Spiking Recovery**

Serum samples were spiked with different amounts of TFF3 and assayed.

<i>Sample</i>	<i>Observed (ng/ml)</i>	<i>Expected (ng/ml)</i>	<i>Recovery O/E (%)</i>
1	1.56	-	-
	4.78	4.56	104.8
	3.10	3.06	101.3
	2.25	2.31	97.4
2	2.11	-	-
	5.29	5.11	103.5
	3.71	3.61	102.8
	3.04	2.86	106.3

• **Linearity**

Serum samples were serially diluted with Dilution Buffer and assayed.

<i>Sample</i>	<i>Dilution</i>	<i>Observed (ng/ml)</i>	<i>Expected (ng/ml)</i>	<i>Recovery O/E (%)</i>
1	-	16.24	-	-
	2x	8.24	8.12	101.5
	4x	3.52	4.06	86.7
	8x	1.96	2.03	96.6
2	-	22.08	-	-
	2x	10.28	11.04	93.1
	4x	5.56	5.52	100.7
	8x	2.52	2.76	91.3

- **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	0.87	0.88	0.73	0.89
2	0.84	0.82	0.66	0.85
3	0.94	0.91	0.77	0.95
4	0.83	0.80	0.67	0.86
5	0.72	0.73	0.57	0.71
6	0.82	0.76	0.63	0.83
7	1.17	1.16	0.96	1.2
8	0.82	0.78	0.64	0.87
9	1.02	1.11	0.82	1.14
10	0.78	0.75	0.60	0.78
<b>Mean (ng/ml)</b>	<b>0.88</b>	<b>0.87</b>	<b>0.70</b>	<b>0.91</b>
<b>Mean Plasma/Serum (%)</b>	-	<b>99</b>	<b>80</b>	<b>103</b>
<b>Coefficient of determination R<sup>2</sup></b>	-	<b>0.928</b>	<b>0.983</b>	<b>0.958</b>

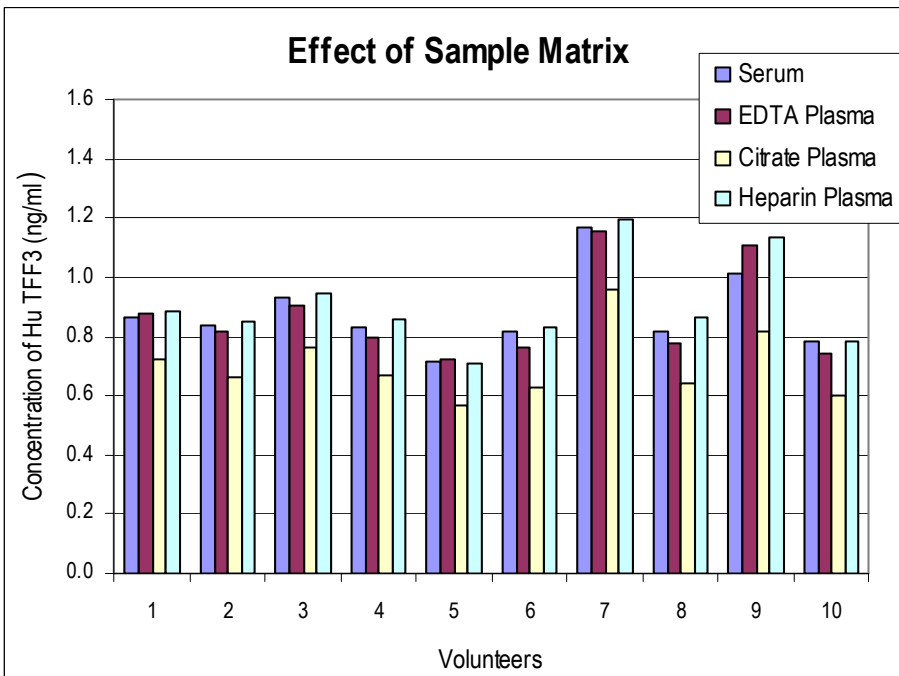


Figure 3: TFF3 levels measured using Human Trefoil Factor 3 ELISA from 10 individuals using serum, EDTA, citrate and heparin plasma, respectively.



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

Samples should be stored at -20°C. However, no decline in concentration of TFF3 was observed in serum and plasma samples after 7 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	1.04	0.98	0.79	1.00
	2-8°C, 1 day	0.91	0.96	0.75	0.98
	2-8°C, 7 days	1.05	0.98	0.82	1.00
2	-20°C	0.98	0.96	0.74	0.97
	2-8°C, 1 day	1.04	1.00	0.85	1.12
	2-8°C, 7 days	1.03	1.14	0.90	1.14
3	-20°C	1.70	1.63	1.41	1.73
	2-8°C, 1 day	1.54	1.64	1.35	1.74
	2-8°C, 7 days	1.66	1.71	1.38	1.73

- **Effect of Freezing/Thawing**

No decline was observed in concentration of human TFF3 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	1.12	1.17	0.77	1.25
	3x	1.10	1.17	0.78	1.32
	5x	1.00	1.19	0.94	1.16
2	1x	1.18	1.27	0.97	1.20
	3x	1.26	1.33	0.93	1.23
	5x	1.29	1.20	0.95	1.44
3	1x	0.90	1.10	0.81	1.16
	3x	1.18	1.18	0.88	1.22
	5x	0.94	1.14	0.86	1.24

## 14. DEFINITION OF THE STANDARD

The recombinant human TFF3 is used as the Standard. The recombinant human TFF3 (AA 1 – 69), produced in *E.coli*, is 7.82 kDa protein containing 59 amino acid residues of the human TFF3 and 10 extra AA.

## 15. PRELIMINARY POPULATION AND CLINICAL DATA

The following results were obtained when serum samples from 119 unselected donors (70 men + 48 women) 21-65 years old were assayed with the Biovendor Human Trefoil Factor 3 ELISA in our laboratory.

- **Age dependent distribution of TFF3**

Sex	Age (years)	n	Mean	SD	Min.	Max.
			TFF3 (ng/ml)			
Men	23-29	12	1.24	0.32	0.83	1.96
	30-39	23	1.09	0.28	0.58	1.72
	40-49	26	1.14	0.34	0.58	2.32
	50-65	9	1.14	0.23	0.90	1.65
Women	22-29	9	8.42	9.64	1.09	30.05
	30-39	19	4.37	6.02	0.75	23.25
	40-49	16	3.00	4.03	0.63	15.50
	50-61	5	1.28	0.40	0.91	2.00

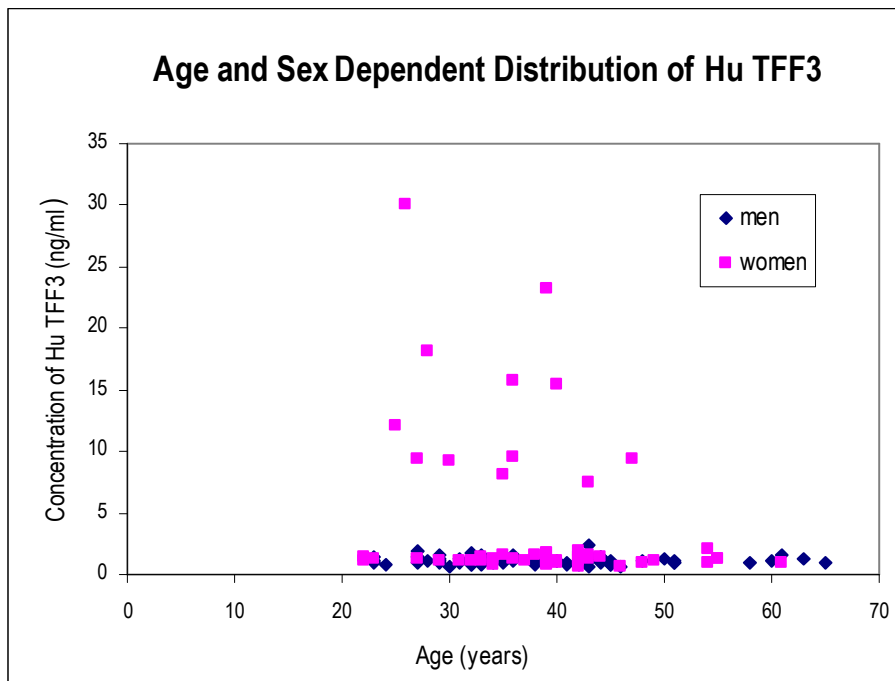


Figure 4: TFF3 concentration plotted against donor age and sex.

- **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 samples in the assay. Each laboratory should establish its own normal and pathological references ranges for TFF3 levels with the assay.

## 16. METHOD COMPARISON

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BioVendor Human Trefoil Factor 3 ELISA has not been compared to any other commercial immunoassay.

## 17. TROUBLESHOOTING AND FAQs

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### »» 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







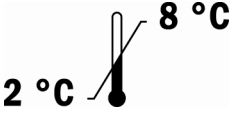

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### »» References to human TFF3:

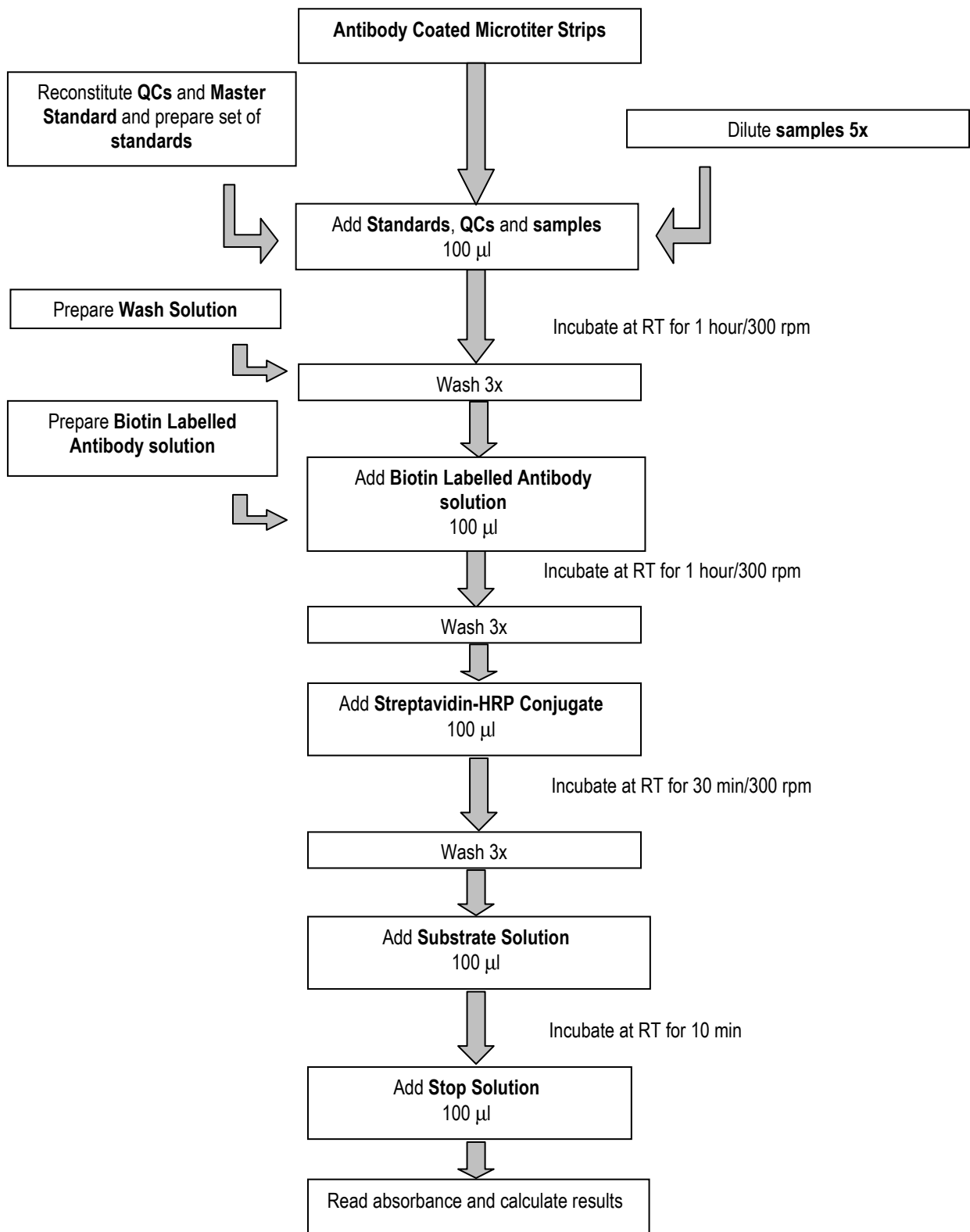
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»» For more references on this product see our WebPages at [www.biovendor.com](http://www.biovendor.com)

## 19. EXPLANATION OF SYMBOLS

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

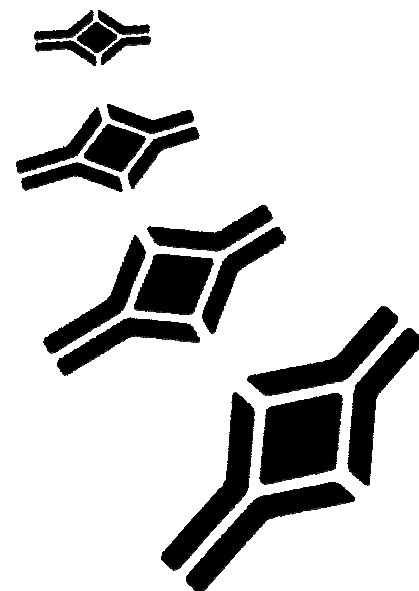
## Assay Procedure Summary



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	<b>A</b>	<b>B</b>	<b>C</b>	<b>D</b>	<b>E</b>	<b>F</b>	<b>G</b>	<b>H</b>

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