Quantikine® ELISA

Human TFF3 Immunoassay

Catalog Number DTFF30

For the quantitative determination of human Trefoil Factor 3 (TFF3) concentrations in cell culture supernates, serum, plasma, saliva, and urine.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Trefoil Factor 3 (TFF3), also known as Intestinal Trefoil Factor (ITF) and P1.B, is one of three structurally related secreted proteins that contain trefoil domains. These domains adopt a three-leaved conformation held together by conserved intrachain disulfide bonds. TFF3 is an approximately 7 kDa peptide that plays an important role in epithelial regeneration and wound healing (1). It can form disulfide-linked dimers or associate into disulfide-linked complexes with the intestinal mucous proteins FCGBP and MUC-2 (2-4). TFF3 is expressed by epithelial goblet cells in the respiratory tract, biliary and breast ducts, conjunctiva, small and large intestine, and cardia of the stomach (5-13). Following secretion, TFF3 is retained in the overlying mucous layer and is also found in breast milk and serum (11, 14, 15). TFF3 is also expressed by chondrocytes during bone development and by select cochlear, hypothalamic, and pituitary neurons (16-18). Mature human TFF3 shares 76% amino acid sequence identity with mouse and rat TFF3 (10, 11).

TFF3 is upregulated in response to a range of epithelial disruptions (6, 19, 20). In the gastrointestinal (GI) tract, it is upregulated in foci of intestinal metaplasia, hyperplastic polyps of the colon, stomach ulceration, and erosive gastro-esophageal reflux disease (GERD) (10, 12, 20). In some cases, it is also elevated in the serum following GI ulceration (15). TFF3 binds to oligosaccharide components of LPS derived from *H. pylori*, a bacterium associated with the development of gastric ulcers and cancer (21). It promotes epithelial wound healing by inducing the migration of biliary, bronchial, and intestinal epithelial cells (7, 19, 22, 23). Mice lacking TFF3 show increased sensitivity to intestinal ulceration and impaired re-epithelialization of the wound (24). Lumenal administration of TFF3 in the intestines can accelerate wound healing and reduce the severity of colitis (24, 25). TFF3 is additionally upregulated in breast and prostate cancers (8, 26), while it is transiently downregulated in the early stages of colon adenocarcinoma (13). Circulating levels of TFF3 are elevated in the serum of patients with advanced prostate cancer (26). Its upregulation is associated with and enhances tumor cell invasion and metastasis (8, 27). TFF3 supports hypoxia-induced VEGF upregulation in tumor cells and also promotes angiogenesis in non-tumor environments (28, 29).

The Quantikine Human TFF3 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human TFF3 in cell culture supernates, serum, plasma, saliva, and urine. It contains CHO cell-expressed recombinant human TFF3 and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human TFF3 showed linear curves that were parallel to the standard curves obtained using the Quantikine kit standards. These results indicate that this kit can be used to determine relative mass values for naturally occurring TFF3.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human TFF3 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any TFF3 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human TFF3 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of TFF3 bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, further dilute the samples with Calibrator Diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
TFF3 Microplate	894421	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against human TFF3.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
TFF3 Conjugate	894422	21 mL of a polyclonal antibody against human TFF3 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
TFF3 Standard	894423	25 ng of recombinant human TFF3 in a buffered protein base with preservatives; lyophilized.	
Assay Diluent RD1-68	895528	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-65	896008	2 vials (21 mL/vial) of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	6 mL of 2 N sulfuric acid.	
Plate Sealers	N/A	4 adhesive strips.	

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 500 mL graduated cylinder.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Test tubes for dilution of standards and samples.
- Human TFF3 Controls (optional; available from R&D Systems).

PRECAUTIONS

TFF3 is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain ProClin® which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Please refer to the MSDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*

Saliva - Collect saliva in a tube and centrifuge for 5 minutes at 10,000 x g. Collect the aqueous layer, and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, and assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Serum and plasma samples require at least a 50-fold dilution. A suggested 50-fold dilution is 10 µL of sample + 490 µL of Calibrator Diluent RD5-65.

Saliva samples require a 500-fold dilution. A suggested 500-fold dilution is 30 µL of sample + 270 µL of Calibrator Diluent RD5-65. Complete the 500-fold dilution by adding 10 µL of the diluted sample to 490 µL Calibrator Diluent RD5-65.

Urine samples require at least a 200-fold dilution. A suggested 200-fold dilution is 30 µL of sample + 270 µL of Calibrator Diluent RD5-65. Complete the 200-fold dilution by adding 25 µL of the diluted sample to 475 µL of Calibrator Diluent RD5-65.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

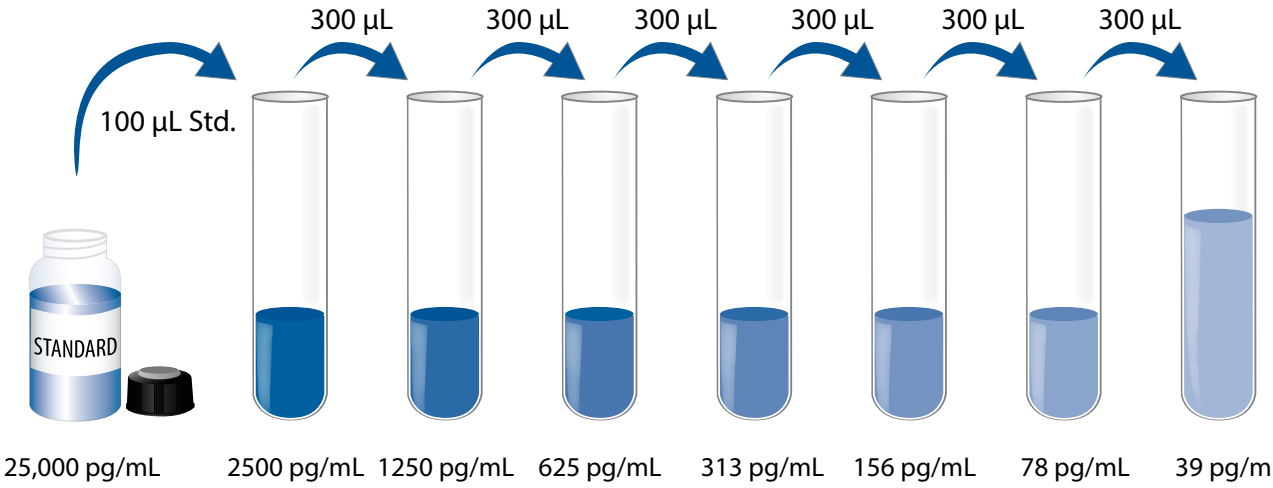
Note: *TFF3 is found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

TFF3 Standard - Reconstitute the TFF3 Standard with 1.0 mL of deionized or distilled water. This reconstitution produces a stock solution of 25,000 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 900 µL of Calibrator Diluent RD5-65 into the 2500 pg/mL tube. Pipette 300 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 2500 pg/mL standard serves as the high standard. Calibrator Diluent RD5-65 serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, controls, and standards be assayed in duplicate.

Note: *TFF3 is found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of Assay Diluent RD1-68 to each well.
4. Add 50 µL of Standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 200 µL of TFF3 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Samples may require dilution. See the Sample Preparation section.

CALCULATION OF RESULTS

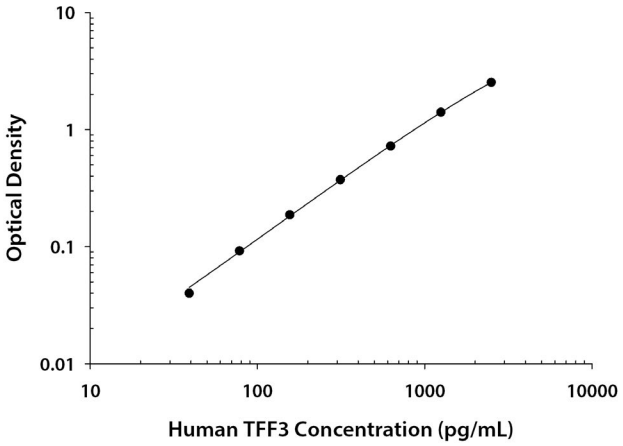
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human TFF3 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

Since samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D.	Average	Corrected
0	0.045 0.047	0.046	—
39	0.084 0.087	0.086	0.040
78	0.135 0.141	0.138	0.092
156	0.230 0.236	0.233	0.187
313	0.418 0.420	0.419	0.373
625	0.765 0.770	0.768	0.722
1250	1.445 1.461	1.453	1.407
2500	2.548 2.601	2.575	2.529

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	276	849	1684	286	894	1712
Standard deviation	6.01	9.13	37.4	18.3	44.0	95.9
CV (%)	2.2	1.1	2.2	6.4	4.9	5.6

RECOVERY

The recovery of human TFF3 spiked to levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	100	90-114%

LINEARITY

To assess the linearity of the assay, samples containing high concentrations of human TFF3 were diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay. Samples were diluted prior to assay.

		Cell culture supernates (n=4)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)	Saliva (n=4)	Urine (n=4)
1:2	Average % of Expected	97	102	102	97	99	104
	Range (%)	89-101	100-105	96-107	93-105	97-101	98-113
1:4	Average % of Expected	98	102	102	97	100	105
	Range (%)	87-108	97-107	99-105	90-105	97-104	102-113
1:8	Average % of Expected	100	100	100	96	102	103
	Range (%)	95-104	96-105	97-105	91-103	97-109	98-108
1:16	Average % of Expected	93	93	96	93	100	96
	Range (%)	86-101	91-97	88-104	89-100	97-104	88-108

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of human TFF3 ranged from 3.10-13.1 pg/mL. The mean MDD was 6.43 pg/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified CHO cell-expressed recombinant human TFF3 manufactured at R&D Systems.

SAMPLE VALUES

Serum/Plasma/Saliva/Urine - Samples from apparently healthy volunteers were evaluated for the presence of human TFF3 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=36)	18.8	8.03-110	22.1
EDTA plasma (n=36)	18.3	7.47-105	21.5
Heparin plasma (n=36)	20.5	6.83-97.0	21.7
Saliva (n=14)	652	136-1674	373
Urine (n=12)	105	23.5-607	162

Cell Culture Supernates:
COLO 205 human colorectal adenocarcinoma cells were cultured in RPMI supplemented with 10% fetal bovine serum. An aliquot of the cell culture supernate was removed, assayed for human TFF3, and measured 19.0 ng/mL.

HT-29 human colon adenocarcinoma cells were cultured in McCoy's media supplemented with 10% fetal bovine serum and 2 mM L-glutamine. An aliquot of the cell culture supernate was removed, assayed for human TFF3, and measured 2.52 ng/mL.

KATO-III human gastric carcinoma cells were cultured in IMDM supplemented with 20% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. An aliquot of the cell culture supernate was removed, assayed for human TFF3, and measured 7.14 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant human TFF3.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range recombinant human TFF3 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

CCL2/MCP-1
CCL5/RANTES
FCGBP
TFF1
TFF2

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