

Datasheet for ABIN578865 Trefoil Factor 2 ELISA Kit



[Go to Product page](#)

Overview

Quantity:	96 tests
Target:	Trefoil Factor 2 (TFF2)
Reactivity:	Pig
Method Type:	Sandwich ELISA
Application:	ELISA

Product Details

Purpose:	This immunoassay kit allows for the in vitro quantitative determination of porcine Intestinal trefoil factor,ITF concentrations in cell culture supernates, tissue homogenates and other biological fluids.
Sample Type:	Cell Culture Supernatant, Tissue Homogenate
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This assay recognizes recombinant and natural porcine ITF.
Cross-Reactivity (Details):	No significant cross-reactivity or interference was observed.
Characteristics:	Sus scrofa,Pig,Trefoil factor 2,Pancreatic spasmolytic polypeptide,PSP,Spasmolytic polypeptide,SP,TFF2,SML1
Components:	Reagent (Quantity): Assay plate (1x20ml), Standard (2), Sample Diluent (1 × 20ml), Assay Diluent A (1x10ml), Assay Diluent B (1x10ml), Detection Reagent A (1 × 120µl), Detection Reagent B (1 × 120µl), Wash Buffer (25 x concentrate) (1 × 30ml), 2 Substrate (1x10ml), Stop Solution (1x10ml), Plate sealer for 96 wells (5), Instruction (1)

Product Details

Material not included: Luminometer. Pipettes and pipette tips. EP tube Deionized or distilled water.

Target Details

Target:	Trefoil Factor 2 (TFF2)
Alternative Name:	TFF2 (TFF2 Products)
Background:	Intestinal trefoil factor (ITF) is a small peptide bearing the unique motif of intrachain disulfide bonds characteristic of the trefoil family. ITF is an essential regulator of colonic epithelial restitution, the rapid migration of colonocytes over mucosal wounds. High levels of ITF are frequently present in colorectal cancers and derived cell lines.
Gene ID:	4099

Application Details

Sample Volume:	100 µL
Plate:	Pre-coated
Protocol:	The microtiter plate provided in this kit has been pre-coated with an antibody specific to ITF. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for ITF and Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB substrate solution is added to each well. Only those wells that contain ITF, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of ITF in the samples is then determined by comparing the O.D. of the samples to the standard curve.
Reagent Preparation:	Bring all reagents to room temperature before use. Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 500 mL of Wash Buffer. Standard - Reconstitute the Standard with 1.0 mL of Sample Diluent. This reconstitution produces a stock solution of 10,000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making serial dilutions. The undiluted standard serves as the high standard (10,000 pg/mL). The Sample Diluent serves as the zero standard (0 pg/mL). Detection Reagent A and B - Dilute to the working concentration specified on the vial label using Assay Diluent A and B (1:100), respectively.

Application Details

Sample Collection: 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 before centrifugation for 15 minutes at approximately 1000 x g. Remove serum and assay immediately or aliquot and store samples at -20 °C. Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000 x g at 2 - 8 °C within 30 minutes of collection. Store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Note: Citrate plasma has not been validated for use in this assay.

Assay Procedure: Allow all reagents to reach room temperature. Arrange and label required number of strips.

1. Prepare all reagents, working standards and samples as directed in the previous sections. 3
2. Add 100 μ L of Standard, Control, or sample* per well. Cover with the adhesive strip. Incubate for 2 hours at 37 °C.
3. Remove the liquid of each well, don't wash.
4. Add 100 μ L of Detection Reagent A to each well. Incubate for 1 hour at 37°C. Detection Reagent A may appear cloudy. Warm to room temperature and mix gently until solution appears uniform.
5. Aspirate each well and wash, repeating the process three times for a total of three washes. Wash by filling each well with Wash Buffer (350 μ L) using a squirt bottle, multi-channel pipette, 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 100 μ L of Detection Reagent B to each well. Cover with a new adhesive strip. Incubate for 1 hours at 37 °C.
7. Repeat the aspiration/wash as in step
5. 8. Add 90 μ L of Substrate Solution to each well. Incubate for 30 minutes at room temperature. Protect from light.
9. Add 50 μ L of Stop Solution to each well. If 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.

Important Note: 1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

2. It is recommended that no more than 32 wells be used for each assay run if manual pipetting is used since pipetting of all standards, specimens and controls should be completed within 5 minutes. A full plate of 96 wells may be used if automated pipetting is available.

- 3. Duplication of all standards and specimens, although not required, is recommended.
- 4. When mixing or reconstituting protein solutions, always avoid foaming.
- 5. 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.
- 6. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.

Calculation of Results: Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density. 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 APC 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. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Restrictions: For Research Use only

Handling

Handling Advice:

- 1. The kit should not be used beyond the expiration date on the kit label.
- 2. Do not mix or substitute reagents with those from other lots or sources.
- 3. If samples generate values higher than the highest standard, further dilute the samples with the Assay 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.
- 4. This assay is designed to eliminate interference by soluble receptors, ligands, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

Storage: 4 °C/-20 °C

Storage Comment: The Standard, Detection Reagent A, Detection Reagent B and the 96-well strip plate should be stored at -20 °C upon being received. The other reagents can be stored at 4 °C.