7th Edition, revised in April, 2017

（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Rat TFF2(Trefoil Factor 2) ELISA Kit

Catalog No: E-EL-R1023

96T

This manual must be read attentively and completely before using this product.

May you have any problems, please contact our Technical Service Center for help.

Phone: 240-252-7368(USA) 240-252-7376(USA)

Email: techsupport@elabscience.com

Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Intended use

This ELISA kit applies to the in vitro quantitative determination of Rat TFF2 concentrations in serum, plasma and other biological fluids.

Specifiction

- Sensitivity: The minimum detectable dose of Rat TFF2 is 37.5pg/mL
- Detection Range : 62.5-4000pg/mL
- Specificity: This kit recognizes natural and some recombinant Rat TFF2. No significant cross-reactivity or interference between Rat TFF2 and analogues was observed.
- Repeatability: Coefficient of variation were<10%.

Test principle

This ELISA kit uses Sandwich-ELISA method. The micro ELISA plate provided in this kit has been pre-coated with antibodies specific to Rat TFF2. Standards or samples added to appropriate micro ELISA plate wells will bind to the antibodies. Biotinylated detection antibodies specific to Rat TFF2 and Avidin-Horseradish Peroxidase (HRP) conjugate can then be added successively to each micro plate well. After incubation, free components are washed away. When the substrate solution is added to each well, only those wells that contain Rat TFF2, biotinylated detection antibody and Avidin-HRP complex will appear blue in color. The enzyme-substrate reaction will be terminated and appears yellow color by adding sulphuric acid solution. The optical density (OD) can be measured with spectrophotometry at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of Rat TFF2. You can calculate the concentration of Rat TFF2 in samples by comparing the OD of the samples with the standard curve.

Kit components & Storage

The unopened kit can be stored at 4 ℃ for 1 week. If the kit is not used within 1 week, store the items separately according to the following conditions.

Item	Specifications	Storage
Micro ELISA Plate(Dismountable)	8 wells ×12 strips	Store at 4 ℃ if it's used within 1 week.
Reference Standard	2 vials	May be stored at -20℃ for 6 month if don't
Concentrated Biotinylated Detection Ab(100×)	1vial 120uL	make recent use
Concentrated HRP Conjugate(100×)	1vial 120μL	May be stored at 4℃(shading light) for 6 months
Reference Standard & Sample Diluent	1vial 20mL	May be stored at 4℃for 6 months
Biotinylated Detection Ab Diluent	1vial 12mL	
HRP Conjugate Diluent	1vial 12mL	
Concentrated Wash Buffer (25×)	1vial 30mL	
Substrate Reagent	1vial 10mL	4℃(shading light)
Stop Solution	1vial 10mL	4℃
Plate Sealer	5pieces	
Product Description	1 copy	
Certificate of Analysis	1 copy	

Note: All reagent bottle cap must be tighten to prevent evaporation and microbial pollution.
The volume of reagents in partial shipments is a little more than the volume marked on the label, please use in measuring instead of directly pouring.

Other supplies required

Microplate reader with 450nm wavelength filter
High-precision transferpettor, EP tubes and disposable pipette tips
37℃ Incubator
Deionized or distilled water
Absorbent paper
Loading slot for Wash Buffer

Note

1. Please wear lab coats and latex gloves for protection. Especially detecting samples of blood or other body fluid, please perform following the national security columns of biological laboratories.
2. The just opened ELISA Plate may appear water-like substance, which is normal and will not have any impact on the experimental results.

3. Don't reuse the diluted standard, biotinylated detection Ab working solution, concentrated HRP conjugate working solution. The unspent undiluted concentrated biotinylated detection Ab(100×) and other stock solution should be stored back according to the storage condition in the above table.
4. The microplate reader should be able to be installed with a filter that can detect the wave length at 450±10nm. The optical density should be within 0~3.5.
5. Do not use components from different batches of kit (washing buffer and stop solution can be an exception).
6. Change pipette tips between adding of each standard level, between sample adding, and between reagent adding. Also, use separate reservoirs for each reagent.

Sample collection

Serum: Allow samples to clot for 2 hours at room temperature or overnight at 4℃ before centrifugation for 15 minutes at 1000×g. Collect the supernatant and carry out the assay immediately. Blood collection tubes should be disposable, non-pyrogenic, and non-endotoxin.

Plasma: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2 - 8℃ within 30 minutes of collection. Collect the supernatant and carry out the assay immediately. Hemolysis samples are not suitable for ELISA assay!

Cell lysates: For adherent cells, gently wash the cells with moderate amount of pre-cooled PBS and dissociate the cells by trypsin. Collect the cell suspension into the centrifugal tube and centrifuge for 5 min at 1000xg. Discard the medium and wash the cells for 3 times with pre-cooled PBS. For each $1x10^6$ cells, add 150-250μL of pre-cooled PBS to keep the cells resuspended. Repeat the freeze-thaw process for several times until the cells are lysed fully. Centrifuge for 10min at 1500xg at 4℃. Remove the cell fragments, collect the supernatant and store at -20℃ or -80℃. Avoid repeated freeze-thaw cycles.

Tissue homogenates: You'd better get detailed references from other literatures before assay aiming at different tissue types. For general information, hemolysis blood may affect the result, so you should mince the tissues to small pieces and rinse them in ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then homogenized in PBS (the volume depends on the weight of the tissue) with a glass homogenizer on ice. To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5 minutes at 50000xg to get the supernatant.

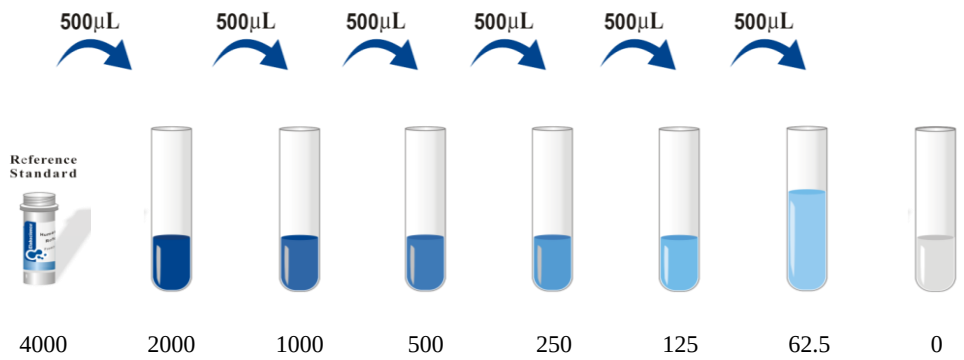
Cell culture supernatant or other biological fluids: Centrifuge samples for 20 minutes at 1000×g at 2 - 8℃. Collect the supernatant and carry out the assay immediately.

Note for sample:

1. Samples should be used within 7 days when stored at 4℃, otherwise samples must be divided and stored at -20℃ (≤1month) or -80℃ (≤3months) .Avoid repeated freeze-thaw cycles.
2. Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments
3. If the sample type is not included in the manual, a preliminary experiment is suggested to vertify the validity.
4. If lysis buffer is used to prepare tissue homogenate or cell culture supernatant, there is a possibility to cause a deviation due to the introduced chemical substance.
5. Some recombinant protein may can't be detected due to the mismatching with coated antibody or detection antibody.

Reagent preparation

1. Bring all reagents to room temperature (18~25℃) before use. Preheat the microplate reader 15 minutes before the assay.
2. **Wash Buffer**: Dilute 30 mL of Concentrated Wash Buffer into 750 mL of Wash Buffer with deionized or distilled water. Note: if crystals have formed in the concentrate, you can warm it with 40℃ water bath and mix it gently until the crystals have completely dissolved.
3. **Standard working solution:** Centrifuge the standard at 10,000×g for 1 minute. Add 1.0mL of Reference Standard &Sample Diluent, let it stand for 10 minutes and turn it upside down for several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a stock solution of 4000pg/mL. Then make serial dilutions as needed. The recommended dilution gradient is as follows: **4000、2000、1000、500、250、125、62.5、0 pg/mL**.
   Dilution method: Take 7 EP tubes, add 500uL of Reference Standard & Sample Diluent to each tube. Pipette 500uL of the 4000pg/mL stock solution to one tube to produce a 2000pg/mL stock solution. Pipette 500uL of the solution from former tube to the latter one in order according to this step. The illustration below is for reference. Note: the last tube is regarded as blank. Don't pipette solution to it from the former tube.



4. **Biotinylated Detection Ab working solution:** Calculate the required amount before experiment (100μL/well). In actual preparation, you should prepare 100~200μL more. Centrifuge the stock tube before use, dilute the 100×concentrated Biotinylated Detection Ab to 1×working solution by Biotinylated Detection Ab Diluent.
5. **Concentrated HRP Conjugate working solution:** Calculate the required amount before experiment (100μL/well). In actual preparation, you should prepare 100~200μL more. Dilute the 100×Concentrated HRP Conjugate to 1×working solution by Concentrated HRP Conjugate Diluent.

Assay procedure(A brief assay procedure is on the 9th page)

1. Add **Standard working solution** of different concentration to the first two columns: Each concentration of the solution is added into two wells side by side(100uL for each well). Add samples to other wells(100uL for each well). Cover the plate with sealer we provided. Incubate for 90 minutes at 37℃. Note: solutions are added to the bottom of micro ELISA plate well, avoid inside wall touching and foaming as possible.
2. Remove the liquid of each well, don't wash. Immediately add 100μL of **Biotinylated Detection Ab working solution** to each well. Cover with the Plate sealer. Gently mix up. Incubate for 1 hour at 37℃.
3. Aspirate or decant the solution from each well，add 350uL of **wash buffer** to each well. Soak for 1~2 minutes and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times. Note: a microplate washer can be used in this step and other wash steps.
4. Add 100μL of **HRP Conjugate working solution** to each well. Cover with the Plate sealer. Incubate for 30 minutes at 37℃.
5. Aspirate or decant the solution from each well, repeat the wash process for five times as conducted in step 3.
6. Add 90μL of **Substrate Reagent** to each well. Cover with a new plate sealer. Incubate for about 15 minutes at 37℃. Protect the plate from light. Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30minutes.
7. Add 50μL of **Stop Solution** to each well. Note: the order to add stop solution should be the same as the substrate solution.
8. Determine the optical density (OD value) of each well at once, using a micro-plate reader set to 450 nm.

Calculation of results

Average the duplicate readings for each standard and samples, then subtract the average zero standard optical density. Create a standard curve by plotting the mean OD value 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. It is recommended to use some professional software to do this calculation, such as curve expert 1.3 or 1.4. In the software interface, a best fitting equation of standard curve will be calculated using OD values and concentrations of standard sample. The software will calculate the concentration of samples after entering the OD value of samples. If samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. If the OD of the sample surpasses the upper limit of the standard curve, you should re-test it after appropriate dilution. The actual concentration is the calculated concentration multiplied dilution factor.

Troubleshooting

Problem	Causes	Solutions
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standard dilution	Ensure briefly spin the vial of standard and dissolve the powder thoroughly by a gentle mix.
	Wells not completely aspirated	Completely aspirate wells between steps.
Low signal	Too brief incubation times	Ensure sufficient incubation time;
	Incorrect assay temperature	Use recommended incubation temperature. Bring substrate to room temperature before use.
	Inadequate reagent volumes	Check pipettes and ensure correct preparation
	Improper dilution	
	HRP conjugate inactive or TMB failure	Mix HRP conjugate and TMB, rapid colouring.
Deep color but low value	Plate reader settings not optimal	Verify the wavelength and filter setting in the plate reader.
		Open the Plate Reader ahead to pre-heat
Large CV	Inaccurate pipetting	Check pipettes
High background	Concentration of detector too high	Use recommended dilution factor.
	Plate is insufficiently washed	Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.
	Contaminated wash buffer	Make fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	All the reagents should be stored according to the instructions
	Stop solution not added	Stop solution should be added to each well before measurement

SUMMARY

1. Add 100μL standard or sample to each well. Incubate 90mintues at 37℃
2. Remove the liquid. Add 100μL Biotinylated Detection Ab. Incubate 1 hour at 37℃
3. Aspirate and wash 3 times
4. Add 100μL HRP Conjugate. Incubate 30 minutes at 37℃
5. Aspirate and wash 5 times
6. Add 90μL Substrate Reagent. Incubate 15 minutes at 37℃
7. Add 50μL Stop Solution. Read at 450nm immediately
8. Calculation of results

Declaration:

1. Limited by current conditions and scientific technology, we can't completely conduct the comprehensive identification and analysis on all the raw material provided. So there might be some qualitative and technical risks for users using the kit.
2. The final experimental results will be closely related to the validity of products, operation skills of the operators and the experimental environments. Please make sure that sufficient samples are available.