OriGene Technologies Inc.
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Mouse TFF1/Ps2 ELISA Kit

Catalog Number:EA102444

Assay Principle

The OriGene Mouse Tff1 Pre-Coated ELISA(Enzyme-Linked Immunosorbent Assay) kit is a solid phase immunoassay specially designed to measure Mouse Tff1 with a 96-well strip plate that is pre-coated with antibody specific for Tff1. The detection antibody is a biotinylated antibody specific for Tff1. The capture antibody is a monoclonal antibody from rat, the detection antibody is a biotinylated polyclonal antibody from goat. The kit contains recombinant Mouse Tff1 with immunogen: Expression system for standard: E.coli, Immunogen sequence: Q22-F87. The kit is analytically validated with ready to use reagents.

To measure Mouse Tff1, add standards and samples to the wells, then add the biotinylated detection antibody. Wash the wells with PBS or TBS buffer, and add Avidin-Biotin-Peroxidase Complex (ABC-HRP). Wash away the unbounded ABC-HRP with PBS or TBS buffer and add TMB. TMB is substrate to HRP and will be catalyzed to produce a blue color product, which changes into yellow after adding acidic stop solution. The density of the yellow product is linearly proportional to Mouse Tff1 in the sample. Read the density of the yellow product in each well using a plate reader, and benchmark the sample wells' readings against the standard curve to determine the concentration of Mouse Tff1 in the sample.

Overview

Product Name	Mouse TFF1/Ps2 ELISA Kit
Reactive Species	Mouse
Size	96wells/kit, with removable strips.
Description	Sandwich High Sensitivity ELISA kit for Quantitative Detection of Mouse TFF1. 96wells/kit, with removable strips.
Sensitivity	<10pg/ml *The sensitivity or the minimum detectable dose (MDD) is the lower limit of target protein that can be detected by the kit. It is determined by adding two standard deviations to the mean O.D. value of twenty (20) blank wells and calculating the corresponding concentration.
Detection Range	31.2pg/ml-2000pg/ml
Storage Instructions	Store at 4°C for 6 months, at -20°C for 12 months. Avoid multiple freeze-thaw cycles(Shipped with wet ice.)
Uniprot ID	Q08423

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Technical Details

Capture/Detection Antibodies	The capture antibody is a monoclonal antibody from rat, the detection antibody is a biotinylated polyclonal antibody from goat.
Specificity	Natural and recombinant Mouse Tff1
Immunogen	Expression system for standard: E.coli, Immunogen sequence: Q22-F87
Cross Reactivity	There is no detectable cross-reactivity with other relevant proteins.

Notice Before Application

Please read the following instructions before starting the experiment.
1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
2. Before using the Kit, spin tubes and bring down all components to the bottom of tubes.
3. Don't let 96-well plate dry, for dry plate will inactivate active components on plate.
4. Don't reuse tips and tubes to avoid cross contamination.
5. Avoid using the reagents from different batches together.

Kit Components/Materials Provided

Description	Quantity	Volume
Anti-Mouse Tff1 Pre-coated 96-well strip microplate	**1**	**12 strips of 8 wells**
Mouse Tff1 Standard	**2**	**10ng/tube**
Mouse Tff1 Biotinylated antibody (100x)	**1**	**100 µl**
Avidin-Biotin-Peroxidase Complex (100x)	**1**	**100 µl**
Sample Diluent	**1**	**30ml**
Antibody Diluent	**1**	**12ml**
Avidin-Biotin-Peroxidase Diluent	**1**	**12ml**
Color Developing Reagent (TMB)	**1**	**10ml**
Stop Solution	**1**	**10ml**
Plate Sealers	**4**	**Piece**

*Why there is no wash buffer? Our Avidin-Biotin-Peroxidase Diluent contains the detergent (TWEEN) normally present in other companies' ELISA kits. This saves you the step of having to wash with the special wash buffer and achieve similar or better signal to noise ratio. The wash can use regular wash buffers (PBS, TBS etc.) commonly found in labs.

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Required Materials That Are Not Supplied

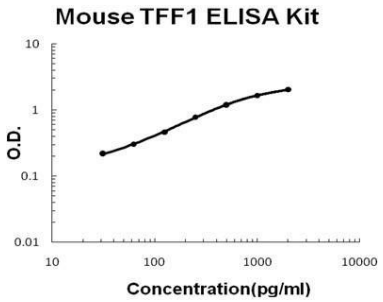
Microplate Reader capable of reading absorbance at 450nm.
Automated plate washer (optional)
1000ml of 1X wash buffer (TBS or PBS)
Pipettes and pipette tips capable of precisely dispensing 0.5 µl through 1 ml volumes of aqueous solutions.
Multichannel pipettes are recommended for large amount of samples.
Deionized or distilled water.
500ml graduated cylinders.
Test tubes for dilution.

Mouse TFF1/Ps2 ELISA Kit (EA102444) Standard Curve Example

Highest O.D. value might be higher or lower than in the example. The experiment result is statistically significant if the highest O.D. value is no less than 1.0.

Concentration (pg/ml)	0	31.2	62.5	125	250	500	1000	2000
O.D.	0.095	0.220	0.305	0.462	0.780	1.196	1.657	2.031

Mouse TFF1 ELISA Kit standard curve



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

Intra/Inter Assay Variability

OriGene spend greatefforts in documenting lot to lot variability and makesureourassaykits produce robust data thatarereproducible.

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

Inter-Assay Precision (Precision across assays): *Three samples of known concentration were tested in separate assays to assess inter-assay*

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precision.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	*1*	*2*	*3*	*1*	*2*	*3*
n	*16*	*16*	*16*	*24*	*24*	*24*
Mean(pg/ml)	*52*	*244*	*807*	*47*	*265*	*781*
Standard deviation	*3.9*	*11.95*	*42.77*	*4.32*	*14.57*	*53.88*
CV(%)	*7.5%*	*4.9%*	*5.3%*	*9.2%*	*5.5%*	*6.9%*

Reproducibility

To assay reproducibility, three samples with differing target protein concentrations were assayed using four different lots.

Lots	*Lot1 (pg/ml)*	*Lot2 (pg/ml)*	*Lot3 (pg/ml)*	*Lot4 (pg/ml)*	*Mean (pg/ml)*	*Standard Deviation*	*CV (%)*
Sample 1	*52*	*60*	*62*	*62*	*59*	*4.12*	*6.9%*
Sample 2	*244*	*254*	*259*	*240*	*249*	*7.59*	*3%*
Sample 3	*807*	*768*	*839*	*743*	*789*	*36.67*	*4.6%*

**number of samples for each test n=16.*

Preparation Before The Experiment

Item	*Preparation*
All reagents	*Bring all reagents to 37°C prior to use. The assay can also be done at room temperature however we recommend doing it at 37°C for best consistency with our QC results. Also the TMB incubation time estimate (15-25min) is based on 37°C.*
Wash buffer	*Prepare 1000ml of 1X PBS or TBS for wash buffer.*
Biotinylated Anti-Mouse Tff1 antibody	*It is recommended to prepare this reagent immediately prior to use by diluting the Mouse Tff1 Biotinylated antibody (100x) 1:100 with Antibody Diluent. Prepare 100 µl by adding 1 µl of Biotinylated antibody (100x) to 99µl ofAntibody Diluent foreachwell. Mixgently and thoroughly andusewithin 2 hours of generation.*
Avidin-Biotin-Peroxidase Complex	*It is recommended toprepare this reagent immediately priortouse by diluting the Avidin-Biotin-Peroxidase Complex (100x) 1:100 with Avidin-Biotin-Peroxidase Diluent. Prepare 100 µl by adding 1 µl of Avidin-Biotin-Peroxidase Complex (100x) to 99 µl of Avidin-Biotin-Peroxidase Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.*
Mouse Tff1 Standard	*It is recommended that the standards be prepared no more than 2 hours prior to performing the*

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	experiment. Use one 10ng of lyophilized Mouse Tff1 standard for each experiment. Gently spin the vial prior to use. Reconstitute the standard to a stock concentration of 10ng/ml using 1ml of sample diluent. Allowthestandard tosit fora minimum of10 minuteswith gentleagitationprior tomakingdilutions.
Microplate	Theincluded microplate is coated with capture antibodiesand ready-to-use. It doesnotrequire additional washing or blocking. Theunusedwellstrips should be sealedand stored in theoriginal packaging.

Dilution of Mouse Tff1 Standard

1. Number tubes 1-8. Final Concentrations to be Tube # 1 –2000pg/ml, #2 –1000pg/ml, #3 – 500pg/ml, #4 – 250pg/ml,#5 – 125pg/ml,#6 – 62.5pg/ml, #7 – 31.25pg/ml, #8 – 0.0 (Blank).
2. To generate standard #1, add 200µl of the reconstituted standard stock solution of 10ng/ml and 800µl of sample diluent to tube #1 for a final volume of 1000µl. Mix thoroughly.
3. Add 300 µl of sample diluent to tubes # 2-7.
4. To generate standard #2, add 300 µl of standard #1 from tube #1 to tube #2 for a final volume of 600 µl. Mix thoroughly.
5. To generate standard #3, add 300 µl of standard #2 from tube #2 to tube #3 for a final volume of 600 µl. Mix thoroughly.
6. Continue the serial dilution for tube #4-7.
7. Tube #8 is a blank standard to be used with every experiment.

Sample Preparation and Storage

These sample collection instructions and storage conditions are intended as a general guideline and the sample stability has not been evaluated.

Sample Type	Procedure
Cell culture supernatants	Clearsample of particulates by centrifugation, assayimmediately or storesamples at-20°C.
Serum	Useaserum separatortube(SST) andallow serum to clot atroom temperature forabout four hours. Then, centrifugefor 15 min at approximately 1,000 xg. assayimmediately or store samples at -20°C.
Plasma	Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 min at approximately 1,000 x g. Assay immediately or store samples at -20°C. *Note: it is important to not use anticoagulants other than the ones described above to treat plasma for other anticoagulants could block the antibody binding site.

Sample Dilution

Thetarget protein concentration should be estimated and appropriate sample dilutions should be selected such that the final protein concentration lies near the middle of the linear dynamic range of the assay.

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It is recommended to prepare 150 µl of sample for each replicate to be assayed. The samples should be diluted with sample diluent and mixed gently.

Assay protocol

It is recommended thatallreagents andmaterials be equilibrated to 37°C/room temperature prior to the experiment(see Preparation Before The Experiment if you have missed this information).

1. Prepare all reagents and working standards as directed previously.
2. Remove excess microplate strips from the plate frame and seal and store them in the original packaging.
3. Add 100 µl of the standard, samples, or control per well. Add 100 µl of the sample diluent buffer into the control well (Zero well). At least two replicates of each standard, sample, or control is recommended.
4. Cover with the plate sealer provided and incubate for 120 minutes at RT (or 90 min. at 37 °C).
5. Remove the cover and discard the liquid in the wells into an appropriate waste receptacle. Invert the plate on the benchtop onto a paper towel and taptheplatetogently blotany remaining liquid. It is recommended thatthewellsare not allowedto completely dry at any time.
6. Add 100 µl of the prepared 1x Biotinylated Anti-Mouse Tff1 antibody to each well.
7. Cover with plate sealer and incubate for 90 minutes at RT (or 60 minutes at 37°C).
8. Wash the plate 3 times with the 1x wash buffer.
a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
b. Add 300 µl of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).
c. Repeat steps a-b 2 additional times.
9. Add 100 µl of the prepared 1x Avidin-Biotin-Peroxidase Complex into each well. Cover with the plate sealer provided and incubate for 40 minutes at RT (or 30 minutes at 37°C).
10. Wash the plate 5 times with the 1x wash buffer.
a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
b. Add 300 µl of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).
c. Repeat steps a-b 4 additional times.
11. Add 90 µl of Color Developing Reagent toeachwell. Cover with theplatesealerprovided and incubate in thedarkfor 30 minutes at RT (or 15-25 minutes at 37°C). (The optimal incubation time mustbe empirically determined. A guideline to look for is blue shading thetop four standard wells, while the remaining standards remain clear.)
12. Add 100 µl of Stop Solution to each well. The color should immediately change to yellow.
13. Within 30 minutes of stopping the reaction, the O.D. absorbance should be read with a microplate reader at 450nm.

Data Analysis

Average the duplicate readings for each standard, sample, and control. Subtract the average zero standard O.D. reading.

It is recommended that a standard curve be created using computer software to generate a four parameter logistic (4-PL) curve-fit. A free program capable of generating a four parameter logistic (4-PL) curve-fit can be found online at: www.myassays.com/four-parameter-logistic-curve.assay.

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Alternatively, plot the mean absorbance for each standard against the concentration. The measured concentration in the sample can be interpolated by using linear regression of eachaverage relative ODagainst the standard curve generated using curve fitting software. This will generate an adequate but less precise fit of the data.

For diluted samples, the concentration reading from the standard curve must be multiplied by the dilution factor.

Background on Tff1

TFF1(Trefoil factor 1), also known as pS2, is a protein that in humans is encoded by the TFF1 gene. Members of the trefoil family are characterized by having at least one copy of the trefoil motif, a 40-amino acid domain that contains three conserved disulfides. They are stable secretory proteins expressed in gastrointestinal mucosa. Their functions arenotdefined, but they may protect themucosafrom insults, stabilize the mucus layer, and affect healing of the epithelium. It is found that TFF1 in normal human urine inhibited the growth of calcium oxalate crystals. Urinary TFF1 showed an inhibitory potency similar to that of nephrocalcin, and inhibition was dose dependent and inhibited by TFF1 antisera, particularlyby antisera directedtothe TFF1Cterminus. Concentrations andrelative amounts ofTFF1in theurine ofpatients with idiopathic calcium oxalate kidney stones were significantly less than those found in controls. This gene, which is expressed in the gastric mucosa, has also been studied because of its expression in human tumors. This gene and two other related trefoil family member genes are found in a cluster on chromosome 21.