SunLong Biotech Co.,LTD

Tel: 0086-571- 56623320 Fax:0086-571- 56623318

E-mail:sales@sunlongbiotech.com

www.sunlongbiotech.com

Quick Step Porcine thrombomodulin,TM ELISA Kit

Size: 96 T, 48T

Catalogue Number:QS0137Po

Assay Time: 60 minutes

Store all reagents at 2-8℃/-20℃

Validity Period: 2-8℃ for six months, -20°C for one year. Avoid repeated thaw cycles.

For samples: In serum, plasma, culture media or any biological fluid.

FOR RESEARCH USE ONLY！

NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS！

PLEASE READ THROUGH ENTIRE PROCEDURE BEFORE BEGINNING！

Quick Step Porcine thrombomodulin,TM ELISA Kit

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Purpose

Our Quick Step Porcine thrombomodulin,TM ELISA Kit is to assay TM levels in Porcine serum, plasma, culture media or any biological fluid.

Principle

This ELISA kit uses Sandwich-ELISA as the method. The Microelisa stripplate provided in this kit has been pre-coated with an antibody specific to TM. Standards or samples are added to the appropriate Microelisa stripplate wells and combined to the specific antibody. Then a Horseradish Peroxidase (HRP)- conjugated antibody specific for TM is added to each Microelisa stripplate well and incubated. Free components are washed away. The TMB substrate solution is added to each well. Only those wells that contain TM and HRP conjugated TM antibody will appear blue in color and then turn yellow after the addition of the stop solution. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The OD value is proportional to the concentration of TM. You can calculate the concentration of TM in the samples by comparing the OD of the samples to the standard curve.

Materials provided with the kit

	Materials provided with the kit	96 determinations	48 determinations
1	User manual	1	1
2	Closure plate membrane	2	2
3	Sealed bags	1	1
4	Microelisa stripplate	1	1
5	Standard:13.5ng/ml	0.5ml×1 bottle	0.5ml×1 bottle
6	Standard diluent	1.5ml×1 bottle	1.5ml×1 bottle
7	HRP-Conjugate reagent	6ml×1 bottle	3ml×1 bottle
8	Sample diluent	6ml×1 bottle	3ml×1 bottle

9	Chromogen Solution A	6ml×1 bottle	3ml×1 bottle
10	Chromogen Solution B	6ml×1 bottle	3ml×1 bottle
11	Stop Solution	6ml×1 bottle	3ml×1 bottle
12	wash solution	20ml (30X)×1bottle	20ml (20X)×1bottle

Sample preparation

1. **Serum preparation**

   After collection of the whole blood, allow the blood to clot by leaving it undisturbed at room temperature. This usually takes 10-20 minutes. Remove the clot by centrifuging at 2,000-3,000 rpm for 20 minutes. If precipitates appear during reservation, the sample should be centrifugated again.

2. **Plasma preparation**

   Collect the whole blood into tubes with anticoagulant (EDTA or citrate). After incubated at room temperature for 10-20 minutes, tubes are centrifugated for 20 min at 2,000-3,000 rpm. Collect the supernatant carefully as plasma samples. If precipitates appear during reservation, the sample should be centrifugated again.

3. **Urine samples**

   Collect urine into aseptic tubes. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If precipitates appear during reservation, the sample should be centrifugated again. The preparation procedure of cerebrospinal fluid and pleuroperitoneal fluid is the same as that of urine sample.

4. **Cell samples**

   If you want to detect the secretions of cells, collect culture supernatant into aseptic tubes. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If you want to detect intracellular components, dilute the cells to 1X100/ml with PBS (pH 7.2-7.4). The cells were destroyed to release intracellular components by repeated freezing and thawing. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If precipitates appear during reservation, the sample should be centrifugated again.

5. **Tissue samples**

   Tissue samples are cut, weighed, frozen in liquid nitrogen and stored at -80℃ for future use. The tissue samples were homogenized after adding PBS (pH 7.4). Samples should be

operated at 4 ℃ . Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. Aliquot the supernatant for ELISA assay and future use.

Notes:

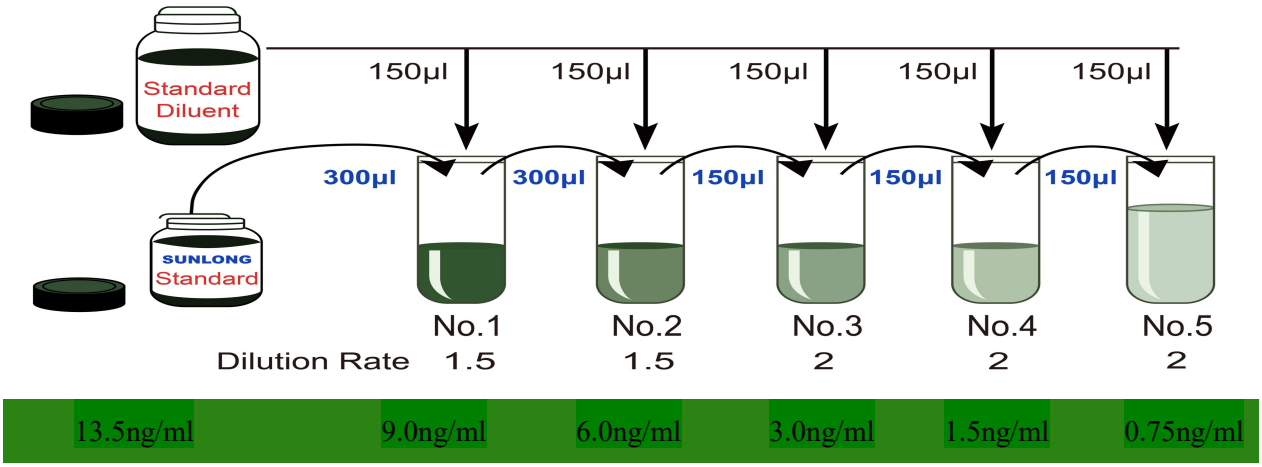
1. Sample extraction and ELISA assay should be performed as soon as possible after sample collection. The samples should be extracted according to the relevant literature. If ELISA assay can not be performed immediately, samples can be stored at -20 ℃ .Repeated freeze-thaw cycles should be avoided.
2. Our kits can not be used for samples with NaN3 which can inhibit the activity of HRP.

"The sample cannot be diluted with this kit. Due to the material preparation kit we use, sample matrix interference may falsely reduce the specificity and accuracy of the detection."

Procedure

1. **Dilution of Standards:**Dilute the standard by small tubes first,then pipette the volume of 50ul from each tube to microplate well,each tube use two wells ,total ten wells.

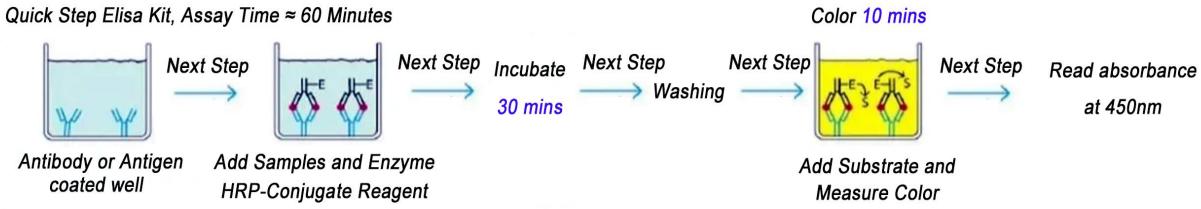
9. 0ng/ml	Standard No.1	300μl Original Standard + 150μl Standard diluents
6. 0ng/ml	Standard No.2	300μl Standard No.1 + 150μl Standard diluents
3. 0ng/ml	Standard No.3	150μl Standard No.2 + 150μl Standard diluent
1. 5ng/ml	Standard No.4	150μl Standard No.3 + 150μl Standard diluent
0. 75ng/ml	Standard No.5	150μl Standard No.4 + 150μl Standard diluent



2. **Add sample:** In the Microelisa stripplate, leave a well empty as blank control. In sample wells, 40μl Sample dilution buffer and 10μl sample are added (dilution factor is 5). Samples should be loaded onto the bottom without touching the well wall. Mix well with gentle shaking.
3. **Add HRP:** Add 50μl HRP-Conjugate reagent to each well except the blank control well.

4. **Incubation:** incubate 30 min at 37℃ after sealed with Closure plate membrane.
5. **Preparing solution:** Dilute the concentrated Wash Solution with distilled water (30 times for 96T and 20 times for 48T).
6. **Washing:** Carefully peel off Closure plate membrane, aspirate and refill with Wash Solution (350μl to 400μl, or fill it completely, overflow is acceptable) .Remove the Wash Solution after resting for 30 seconds. Repeat the washing procedure for 5 times. After the last wash, remove any remaining Wash Solution by aspirating or decanting. Invert the plate and blot it against clean paper towels.
7. **Chromogenic reaction:** Add 50 μl Chromogen Solution A and 50μl Chromogen Solution B to each well, mix with gently shaking and incubate at 37℃ for 10min. Protect from light.
8. **Stop the reaction:** Add 50μl Stop Solution to each well to terminate the reaction. The color in the wells should change from blue to yellow.
9. **Assay:** Read absorbance O.D. at 450nm using a Microtiter Plate Reader. The OD value of the blank control well is set as zero. Assay should be carried out within 15min after adding stop solution.

Summary:



Notes:

1. Store the kit at 2-8℃/-20° C upon receipt.The kit should be equilibrated to room temperature before the assay. Remove any unneeded strips from Porcine TM antibody-Coated plate, reseal them in zip-lock foil and keep at 2-8℃/-20° C.
2. Precipitates may appear in concentrated washing buffer. Please heat the buffer to dissolve all the precipitates, which will not affect the results.
3. Accurate pipette should be used to avoid experimental error. Samples should be added to the Microplate in less than 5 minutes. If a large number of samples are included, multiple

channel pipette is recommended.

4. Standard curve should be included in every assay. Replicate wells are recommended. If the OD value of the sample is greater than the first well of standards, please dilute the sample (n times) before test. When calculating the original TM concentration, please multiply the total dilution factor (XnX5).
5. In order to avoid cross-contamination, Microplate sealers are for one-time use only.
6. Please keep Substrate away from light.
7. All the operation should be accordance with the manufacturer's instructions strictly. The results determined by the Microtiter Plate Reader.
8. All the samples, washing buffer and wastes should be treated as infectious agents.
9. Reagents from different lots should not be mixed.

Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level Porcine TM were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level Porcine TM were tested on 3 different plates, 8 replicates in each plate.

CV(%) = SD/meanX100

Intra-Assay: CV<10%

Inter-Assay: CV<12%

Assay range

0.1ng/ml-10ng/ml

Sensitivity

0.01ng/ml

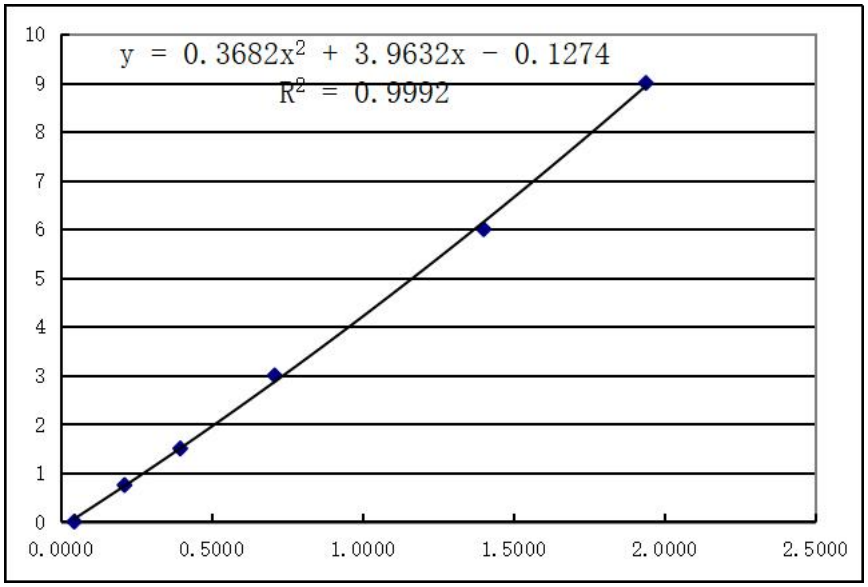
Calculation of Results

Known concentrations of Porcine TM Standard and its corresponding reading OD is plotted on the log scale (x-axis) and the log scale (y-axis) respectively. The concentration of Porcine TM in sample is determined by plotting the sample’s O.D. on the Y-axis. The original concentration is calculated by multiplying the dilution factor.

Equation: Polynomial Quadratic Regression

Typical Data

The standard curve of QS0137Po is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



Standard	Concentration	OD Value	Average OD Value
Blank Well	0ng/ml	0.043	0.0460
		0.049	
S1	9ng/ml	1.933	1.9385
		1.944	
S2	6ng/ml	1.394	1.4015
		1.409	
S3	3ng/ml	0.713	0.7095
		0.706	
S4	1.5ng/ml	0.400	0.3970
		0.394	
S5	0.75ng/ml	0.206	0.2125

		0. 219	

Troubleshooting

Weak Signal	**Solution**
Improper washing	Increasing duration of soaking steps
Incorrect incubation temperature	Incubate at room temperature
antibody are not enough	Increase the concentration of the antibody
Reagent are contaminated	Use new one
Pipette are not clean	Pipette should be clean
No Signal	**Solution**
Reagent are contaminated	Use new one
Sample prepared incorrectly	Make sure the sample workable/dilution
antibody are not enough	Increase the antibody concentration
Wash buffer contains sodium azide	Use a new wash buffer and avoid sodium azide in it
HRP was not added	Add HRP according to the instruction
Poor Precision	**Solution**
Imprecise/ inaccurate pipetting	Check/ calibrate pipettes
Incomplete washing of the wells	Make sure wells are washed adequately by filling the wells with wash buffer and all residual antibody solutions crossed well before washing.