Taiclone®
Quality Guarantee

USER MANUAL



ELISA KIT

ELISA Kit for Translocator Protein (TSPO)

Catalog No.tcue3393 96 Tests

FOR RESEACH USE ONLY!

Please read completely user manual and storage condition.

Taiclone®
Quality Guarantee

Web: www.taiclone.com
Tel: +886-2-2735-9682
Email: order@taiclone.com

Human Translocator Protein (TSPO) ELISA Kit

Catalog No.tcue3393

Available Sizes

Size: 96 Tests

Specifications

Application:	For quantitative detection of TSPO in serum, plasma, tissue homogenates and other Biological fluids
Species Reactivity:	Human
Sample Type:	Serum, plasma, Cell Culture supernatant,tissue Homogenates, other biological fluids
Sensitivity:	< 0.113ng/mL
Recovery:	Recovery ranges are from 93-98% .
Detection Range:	0.312-20ng/mL
Assay Time:	3hrs
Detection Method:	Sandwich ELISA, Double Antibody,Colorimetric; absorbance at 450 nm
Precision:	Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level Human TSPO were tested 20 times on one plate, respectively. Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level Human TSPO were tested on 3 different plates, 8 replicates in each plate. Intra-assay coefficient of variation (CV) <10%. Inter-assay CV <10%.

Test Principle

This kit was based on sandwich enzyme-linked immune-sorbent assay technology.
Anti- TSPO antibody was pre-coated onto 96-well plates.
And the biotin conjugated anti-TSPO antibody was used as detection antibodies.
The standards, test samples and biotin conjugated detection antibody were added to the wells subsequently, and wash with wash buffer.
HRP Streptavidin was added and unbound conjugates were washed away with wash buffer.
TMB substrates were used to visualize HRP enzymatic reaction.
TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the TSPO amount of sample captured in plate. Read the O.D. absorbance at 450nm in a microplate reader, and then the concentration of TSPO can be calculated..

Web: www.taiclone.com
Tel: +886-2-2735-9682
Email: order@taiclone.com

Materials and Storage.

Store kit components of Standard, Detection,Reagent A, Detection Reagent B and the 96-well strip plate at -20 °C , Others reagent store at 4 °C ..
DO NOT USE past kit expiration date. Some vials contain a small amount of reagents.
Spin tubeson pulse setting prior to opening.

Opened and used Kit.

All kits component should be stored at its appropriate temperature storage.
Must be used within ONE month after opening.

Components

Reagents	Quantity	Reagents	Quantity
Pre-coated, ready to use 96-well strip plate	1	Plate sealer for 96 wells	4
Standard	2	Standard Diluent	1×20mL
Detection Reagent A	1×120μL	Assay Diluent A	1×12mL
Detection Reagent B	1×120μL	Assay Diluent B	1×12mL
TMB Substrate	1×9mL	Stop Solution	1×6mL
Wash Buffer (30 × concentrate)	1×20mL	Instruction manual	1

Materials and instruments required but not supplied

- Precision pipettes calibrated to deliver 5-1000μL
- Multi-channel pipette calibrated to deliver 50-250μL
- Plate shaker
- Disposable tips
- Vortex-Mixer
- Distilled or de-ionized water
- Microplate reader capable of reading 450nm.

Safety Precautions

- The test protocol must be followed strictly.
- All reagents containing human material should be handled as if potentially infectious. Operators should wear gloves and protective clothing when handling any patient sera or serum based products.
- The kit reagents contain antimicrobial agents, acid and 3,3',5,5'-tetramethylbenzidine. Avoid contact with the skin and eyes. Rinse immediately with plenty of water if any contact occurs.
- Any liquid that has been brought into contact with potentially infectious material has to be discarded in a container with a disinfectant. Disposal must be performed in accordance with local regulations.
- Disposal must be performed in accordance with local regulations.
- Only trained laboratory personnel should execute this test.

Web: www.taiclone.com
Tel: +886-2-2735-9682
Email: order@taiclone.com

Serum: Allow samples to clot for 2 hours at room temperature or overnight at 4°C before centrifugation for 15 min at 1000×g at 2~8°C. Collect the supernatant to carry out the assay. Blood collection tubes should be disposable and be nonendotoxin.

Plasma: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 min at 1000×g at 2~8°C within 30 min of collection. Collect the supernatant to carry out the assay. Hemolysed 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 cellsusing trypsin. Collect the cell suspension into a centrifuge tube and centrifuge for 5 min at 1000×g. Discard the medium and wash the cells 3 times with pre-cooled PBS. For each 1×10^6 cells, add 150-250 μL of pre-cooled PBS to keep the cells suspended. Repeat the freeze-thaw process several times until the cells are fully lysed. Centrifuge for 10min at 1500×g at 4°C. Remove the cell fragments, collect the supernatant to carry out the assay. Avoid repeated freeze-thaw cycles.

Tissue homogenates: It is recommended to get detailed references from the literature before analyzing different tissue types. For general information, hemolysed blood may affect the results, so the tissues should be minced into small pieces and rinsed in ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then homogenized in PBS (tissue weight (g): PBS (mL) volume=1:9) with a glass homogenizer on ice. To further break down 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 min at 5000×g to get the supernatant.

Cell culture supernatant or other biological fluids: Centrifuge samples for 20 min at 1000×g at 2~ 8°C. Collect the supernatant to carry out the assay.

Note for sample:

1. Samples should be assayed within 7 days when stored at 4°C, otherwise samples must be divided up and stored at -20°C (≤1 month) or -80°C (≤3 months). 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 verify the validity.
4. If a lysis buffer is used to prepare tissue homogenates or cell culture supernatant, there is a possibility of causing a deviation due to the introduced chemical substance.
5. Some recombinant protein may not be detected due to a mismatching with the coated antibody or detection antibody.

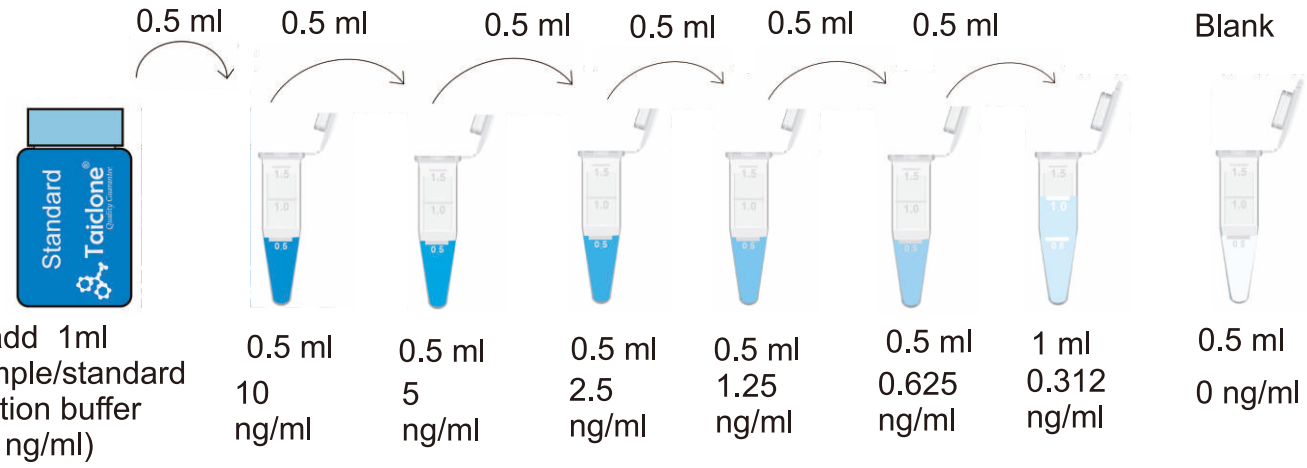
Web: www.taiclone.com
Tel: +886-2-2735-9682
Email: order@taiclone.com

Reagent preparation

1. Bring all reagents to room temperature (18~25°C) before use.
   Follow the Microplate reader manual for set-up and
   preheat it for 15 min before OD measurement.
2. Wash Buffer: Dilute 30 mL of Concentrated Wash Buffer with 720 mL of deionized or distilled water to prepare 750 mL of Wash Buffer.

Note: if crystals have formed in the concentrate, warm it in a 40°C 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 min.
   Add 1.0 mL of Reference Standard &Sample Diluent, let it stand for 10 min and invert it gently several times. After it dissolves fully, mix it thoroughly with a pipette.
   This reconstitution produces a working solution of 20 ng/mL.
   Then make serial dilutions as needed.
   The recommended dilution gradient as follows: 20, 10, 5, 2.5, 1.25, 0.625, 0.312, 0 ng/mL.
   Dilution method: Take 7 EP tubes, add 500uL of Reference Standard & Sample Diluent to each tube. Pipette 500uL of the 20 ng/mL working solution to the first tube and mix up to produce a 10 ng/mL working solution.
   Pipette 500uL of the solution from the former tube into the latter one according to these steps.
   The illustration below is for reference. Note: the last tube is regarded as a blank.
   Don't pipette solution into it from the former tube.



4. Detection Reagent A to working solution: Calculate the required amount before the experiment (100 µL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the stock tube before use, dilute the 100× Reagent A by using Assay Diluent A to 1×working solution.
5. Detection Reagent B working solution: Calculate the required amount before the experiment (100 µL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the stock tube before use, Dilute the 100× Reagent B by using Assay Diluent B to 1× working solution .

Web: www.taiclone.com
Tel: +886-2-2735-9682
Email: order@taiclone.com

Assay procedure

1. Add the Standard working solution to the first two columns: Each concentration of the solution is added in duplicate, to one well each, side by side (100 uL for each well). Add the samples to the other wells (100 uL for each well). Cover the plate with the sealer provided in the kit. Incubate for 60 min at 37°C. Note: solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible.
2. OPEN the seal cover and Remove the liquid out of each well, pat the Plate on Absorbent paper. DO NOT WASH PLATE (DO NOT LET DRY.)
3. Immediately add 100 μL of Detection Reagent A solution to each well. Cover with the Plate sealer. Gently mix up. Incubate for 1 hour at 37°C.
4. Aspirate or decant the solution from each well, add 350 uL of wash buffer to each well. Soak for 1~2 min 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.
5. Add 100 μL of Detection Reagent B working solution to each well. Cover with the Plate sealer. Incubate for 30 min at 37°C.
6. Aspirate or decant the solution from each well, repeat the wash process for five times as conducted in step 4.
7. Add 90 μL of Substrate Solution to each well. Cover with a new plate sealer. Incubate for about 10 ~20min at 37°C. Protect the plate from light. Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30min.
8. Add 50 μL of Stop Solution to each well. Note: Adding the stop solution should be done in the same order as the substrate solution.
9. Determine the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm

Calculation of results

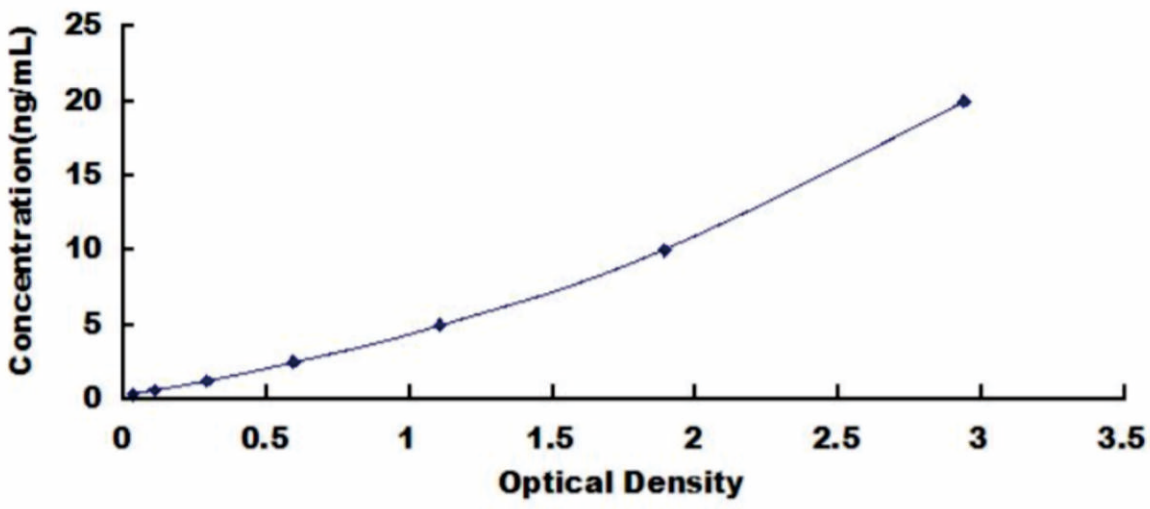
Average the duplicate readings for each standard and samples, then subtract the average zero standard optical density. Plot a four-parameter logistic curve on log-log graph paper, with standard concentration on the x-axis and OD values on the y-axis.

If the 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 with an appropriate dilution. The actual concentration is the calculated concentration multiplied by the dilution factor.

Typical data

As the OD values of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test. Typical standard curve and data is provided below for reference only.

X	pg/ml	0	46.875	93.75	187.5	375	750	1500	3000
Y	OD450	0.028	0.054	0.077	0.153	0.27	0.542	1.112	2.172



Typical Standard Curve for TSPO, Human ELISA.

Web: www.taiclone.com
Tel: +886-2-2735-9682
Email: order@taiclone.com

Specificity

This assay has high sensitivity and excellent specificity for detection of TSPO .
No significant cross-reactivity or interference between TSPO and analogues was observed.
Note: Limited by current skills and knowledge, it is difficult for us to complete the crossreactivity detection between TSPO and all the analogues, therefore, cross reaction may still exist.

To minimize extra influence on performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly controlled.
It is strongly suggested that the same operator performs the whole assay from the beginning to the end.

Web: www.taiclone.com
Tel: +886-2-2735-9682
Email: order@taiclone.com

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 gentle mixing.
	Wells are not completely aspirated	Completely aspirate wells in between steps.
Low signal	Insufficient incubation time	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 coloring.
Deep color but low value	Plate reader setting is not optimal	Verify the wavelength and filter setting on the Microplate reader.
		Open the Microplate Reader ahead to pre-heat.
Large CV	Inaccurate pipetting	Check pipettes.
High background	Concentration of target protein is 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	Prepare fresh wash buffer.
Low sensitivity	Improper storage of the ELISA kit	All the reagents should be stored according to the instructions.
	Stop solution is not added	Stop solution should be added to each well before measurement.

Web: www.taiclone.com
Tel: +886-2-2735-9682
Email: order@taiclone.com

SUMMARY

1. Add 100 μL standard or sample to each well. Incubate for 60 min at 37°C.
2. Remove the liquid. Add 100 μL Detection Reagent A. Incubate for 1 hour at 37°C.
3. Aspirate and wash 3 times.
4. Add 100 μL Detection Reagent B Incubate for 30 min at 37°C.
5. Aspirate and wash 5 times.
6. Add 90 μL Substrate Solution and Incubate for 15 min at 37°C.
7. Add 50 μL Stop Solution. Read at 450 nm immediately.
8. Calculation of results.

Declaration

1. Limited by current conditions and scientific technology, we can't conduct 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, operational skills of the operators and the experimental environments. Please make sure that sufficient samples are available.
3. To get the best results, please only use the reagents supplied by the manufacturer and strictly comply with the instructions!
4. Incorrect results may occur because of incorrect operations during the reagents preparation and loading, as well as incorrect parameter settings of the Micro-plate reader. Please read the instructions carefully and adjust the instrument prior to the experiment.
5. Even the same operator might get different results in two separate experiments. In order to get reproducible results, the operation of every step in the assay should be controlled.
6. Every kit has strictly passed QC test. However, results from end users might be inconsistent with our data due to some variables such as transportation conditions, different lab equipments, and so on. Intra-assay variance among kits from different batches might arise from the above reasons, too.

Taiclone Biotech Corp.

Tel :+886 2 2735 9682
Fax:+886 2 2735 9807
Email: order@taiclone.com
Website:www.taiclone.com