Human OXB (Orexin B) ELISA Kit

Catalogue No.: EH3488

Size: 96T

Reactivity: Human

Range: 0.391 -25ng/ml

Sensitivity: <0.235ng/ml

Application: For quantitative detection of Human OXB in serum, plasma, tissue homogenates and other biological fluids.

Storage: 1.It's OK to keep the kit in 4℃,if the kit is scheduled to be used up in one week.

2.6 months: (only Micro ELISA Plate, Lyophilized Standard and Concentrated Biotinylated Detection Antibody at -20℃, other Components at 2-8℃)

3.12 months: (if the whole kit is stored at -20℃.Avoid repeated freeze-thaw cycles)

NOTE: FOR RESEARCH USE ONLY.

Kit Components

Item	Specifications(96T)	Storage
Micro ELISA Plate(Dismountable)	8 ×12	4℃/-20℃
Lyophilized Standard	2 vial	4℃/-20℃
Sample / Standard dilution buffer	20ml	4℃
Biotin- detection antibody (Concentrated)	120ul	4℃/-20℃
Antibody dilution buffer	10ml	4℃
HRP-Streptavidin Conjugate(SABC)	120ul	4℃(shading light)
SABC dilution buffer	10ml	4℃
TMB substrate	10ml	4℃(shading light)
Stop solution	10ml	4℃
Wash buffer (25X)	30ml	4℃
Plate Sealer	5pieces	
Product Description	1 copy	

Principle of the Assay

This ELISA kit uses Competitive-ELISA as the method. The microtiter plate provided in this kit has been pre-coated with Human OXB. During the reaction,Human OXB in the sample or standard competes with a fixed amount of Human OXB on the solid phase supporter for sites on the Biotinylated Detection Antibody specific to Human OXB. Excess conjugate and unbound sample or standard are washed from the plate, and HRP-Streptavidin (SABC) is added to each microplate well and incubated. Then a TMB substrate solution is added to each well. 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. The concentration of Human OXB in the samples is then determined by comparing the O.D. of the samples to the standard curve.

Precautions for Use

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.After opening and before using, keep plate dry.
3. Before using the Kit, spin tubes and bring down all components to the bottom of tubes.
4. Storage TMB reagents avoid light.
5. Washing process is very important, not fully wash easily cause a false positive.
6. Duplicate well assay is recommended for both standard and sample testing.
7. Don't let Micro plate dry at the assay, for dry plate will inactivate active components on plate.
8. Don't reuse tips and tubes to avoid cross contamination.
9. Avoid using the reagents from different batches together.

Material Required But Not Supplied

1.Microplate reader (wavelength: 450nm)
2.37℃ incubator
3.Automated plate washer
4.Precision single and multi-channel pipette and disposable tips
5.Clean tubes and Eppendorf tubes
6.Deionized or distilled water

Manual Washing

Discard the solution in the plate without touching the side walls. Clap the plate on absorbent filter papers or other absorbent material. Fill each well completely with 350ul wash buffer and soak for 1 to 2 minutes, then aspirate contents from the plate, and clap the plate on absorbent filter papers or other absorbent material. Repeat this procedure two more times for a total of THREE washes.

Automated Washing

Aspirate all wells, then wash plate THREE times with 350ul wash buffer. After the final wash, invert plate, and clap the plate on absorbent filter papers or other absorbent material. It is recommended that the washer be set for a soaking time of 1 minute.

Sample Collection and Storage

Isolate the test samples soon after collecting, then, analyze immediately (within 2 hours). Or aliquot and store at -20℃ for long term. Avoid multiple freeze-thaw cycles.

- Serum： Allow samples to clot for 2 hours at room temperature or overnight at 4℃ before centrifugation for 20 minutes at approximately 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-Na_2 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. Avoid hemolysis, high cholesterol samples.
- Tissue homogenates : For general information, hemolysis blood may affect the result, so you should rinse the tissues with ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then minced to small pieces which will be homogenized in PBS (the volume depends on the weight of the tissue. 9mL PBS would be appropriate to 1 gram tissue pieces. Some protease inhibitor is recommended to add into the PBS.) 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 centrifugated for 5minutes at 5000× g to get the supernate.
- Cell culture supernate： Centrifuge supernate for 20 minutes to remove insoluble impurity and cell debris at 1000× g at 2 - 8℃. Collect the clear supernate and carry out the assay immediately.

- Other biological fluids : Centrifuge samples for 20 minutes at 1000× g at 2 - 8℃. Collect the supernatant and carry out the assay immediately.
- Sample preparation : Samples should be clear and transparent and be centrifuged to remove suspended solids..

Note: Samples to be used within 5 days may be stored at 4℃,otherwise samples must be stored at -20℃ (≤1 month) or -80℃(≤2 months) to avoid loss of bioactivity and contamination. Hemolyzed samples are not suitable for use in this assay.

Reagent Preparation and Storage

Bring all reagents to room temperature before use.

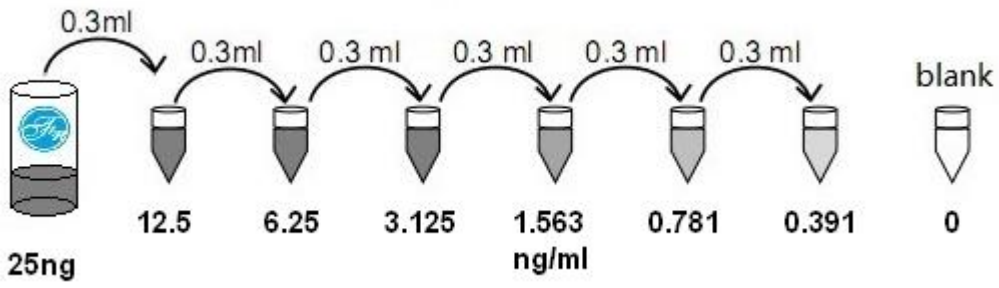
1, Wash Buffer :

Dilute 30mL of Concentrated Wash Buffer into 750 mL of Wash Buffer with deionized or distilled water. Put unused solution back at 4℃. If crystals have formed in the concentrate, you can warm it with 40℃ water bath (Heating temperature should not exceed 50℃) and mix it gently until the crystals have completely dissolved. The solution should be cooled to room temperature before use.

2, Standard :

1). 25 ng/ml of standard solution: Add 1 ml of Sample / Standard dilution buffer into one Standard tube, keep the tube at room temperature for 10 min and mix thoroughly.

2). 12.5 ng/ml →0.391ng/ml of standard solutions: Label 6 Eppendorf tubes with 12.5ng/ml, 6.25 ng/ml, 3.125 ng/ml, 1.563 ng/ml, 0.781ng/ml, 0.391ng/ml, respectively. Aliquot 0.3 ml of the Sample / Standard dilution buffer into each tube. Add 0.3 ml of the above 25 ng/ml standard solution into 1st tube and mix thoroughly. Transfer 0.3 ml from 1st tube to 2nd tube and mix thoroughly. Transfer 0.3 ml from 2nd tube to 3rd tube and mix thoroughly, and so on.



Note: The standard solutions are best used within 2 hours. The standard solution should be at 4℃ for up to12 hours. Or store at -20 ℃ for up to 48 hours. Avoid repeated freeze-thaw cycles.

3. Preparation of Biotin- detection antibody working solution

prepare within 1 hour before the experiment.

1). Calculate the total volume of the working solution: 0.05 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume)

2). Dilute the Biotin- detection antibody with Antibody dilution buffer at 1:100 and mix thoroughly. (i.e. Add 1 µl of Biotin conjugated detection antibody into 99 µl of Antibody dilution buffer.)

4. Preparation of HRP-Streptavidin Conjugate (SABC) working solution:

prepare within 30min before the experiment.

1). Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume)

2). Dilute the SABC with SABC dilution buffer at 1:100 and mix thoroughly. (i.e. Add 1 µl of SABC into 99 µl of SABC dilution buffer.)

Assay Procedure

Before adding to wells, equilibrate the SABC working solution and TMB substrate for at least 30 min at room temperature (37 ℃). When diluting samples and reagents, they must be mixed completely and evenly.It is recommend to plot a standard curve for each test.

1. Set standard, test sample and control (zero) wells on the pre-coated plate respectively, and then, record their positions. It is recommend to measure each standard and sample in duplicate. **Wash plate 2 times before adding standard, sample and control (zero) wells!**

2. **Add Sample and Biotinylated Detection Antibody**: Add 50µL of Standard, Blank, or Sample per well. The blank well is added with Sample / Standard dilution buffer. Immediately add 50 µL of Biotinylated Detection Antibody working solution to each well. Cover with the Plate sealer we provided. Gently tap the plate to ensure thorough mixing. Incubate for 45minutes at 37℃. (Solutions are added to the bottom of micro ELISA plate well, avoid inside wall touching and foaming to the best of your ability.)

3. **Wash**: Aspirate each well and wash, repeating the process three times Wash by filling each well with Wash Buffer (approximately 350µL) using a squirt bottle, multi-channel pipette, manifold dispenser or automated washer. 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 pat it against thick clean absorbent paper.

4. **HRP-Streptavidin Conjugate(SABC)**: Add 100μL of SABC working solution to each well. Cover with a new Plate sealer. Incubate for 30minutes at 37℃.

5. **Wash**: Repeat the aspiration/wash process for five times.

6. **TMB Substrate**: Add 90μL of TMB Substrate to each well. Cover with a new Plate sealer. Incubate for about 15 minutes at 37℃. Protect from light. The reaction time can be shortened or extended according to the actual color change, but not more than 30minutes. When apparent gradient appeared in standard wells, you can terminate the reaction.

7. **Stop**: Add 50μL of Stop Solution to each well. Color turn to yellow immediately. The adding order of stop solution should be as the same as the substrate solution.

8. **OD Measurement**: Determine the optical density (OD Value) of each well at once, using a microplate reader set to 450 nm. You should open the microplate reader ahead, preheat the instrument, and set the testing parameters.

Calculation of results

Average the duplicate readings for each standard and samples. Create a standard curve by plotting the mean OD Value for each standard on the y-axis or x-axis against the concentration on the x-axis or y-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 Value and concentrations of standard sample. The software will calculate the concentration of samples after entering the OD Value of samples. Also, you can enter the corresponding fitting equation and OD Value of samples into Excel to get the concentration 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. Recommended to use professional software curve expert to 1.3,for details, please visit: www.fn-test.com.

Summary

1. Add 50μL standard or sample to each well

2. Immediately add 50μL Biotinylated Detection Antibody to each well
3. Incubate for 45 minutes at 37℃
4. Aspirate and wash 3 times
5. Add 100μL HRP Conjugate to each well. Incubate for 30 minutes at 37℃
6. Aspirate and wash 5 times
7. Add 90μL Substrate Reagent. Incubate 15 minutes at 37℃
8. Add 50μL Stop Solution. Read at 450nm immediately
9. Calculation of results

Typical Data & Standard Curve

Results of a typical standard run of a Human OXB ELISA Kit are shown below. This standard curve was generated at our lab for demonstration purpose only. Each user should obtain their own standard curve as per experiment. (N/A=not applicable)

X	ng/ml	0	0.391	0.781	1.563	3.125	6.25	12.5	25
Y	OD450	2.079	1.885	1.514	1.227	0.877	0.674	0.428	0.360