

Human Orexin receptor type 1 ELISA Kit

Cat #: orb1660780 (manual)

For research use only; not for therapeutic or diagnostic applications!

Please read through entire procedure before beginning!

SYNONYMS

Orexin receptor type 1(Ox-1-R, Ox1-R, Ox1R); Hypocretin receptor type 1

INTENDED USE

This immunoassay kit allows for the in vitro quantitative determination of general target antigen concentrations in serum, plasma, tissue homogenates, cell culture supernates, and other biological fluids.

TEST PRINCIPLE

The microtiter plate provided in this kit has been pre-coated with an antibody specific to target antigen. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated antibody preparation specific for target antigen and then avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB substrate solution is added to each well. Only those wells that contain target antigen, biotin- conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. 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 $\pm$ 2 nm. The concentration of target antigen in the samples is then determined by comparing the O.D. of the samples to the standard curve.

Materials and components

Reagent	Quantity
Assay plate	1
Standard	2
Sample Diluent	1 $\times$ 20mL
Assay Diluent A	1 $\times$ 12mL
Assay Diluent B	1 $\times$ 12mL
Detection Reagent A	1 $\times$ 120 μ L
Detection Reagent B	1 $\times$ 120 μ L
Wash Buffer (25 x concentrate)	1 $\times$ 30mL

Substrate	1 × 10mL
Stop Solution	1 × 10mL
Plate sealer for 96 wells	5
Instruction	1

OTHER SUPPLIES REQUIRED

Microplate reader.

Pipettes and pipette tips.

EP tube

Deionized or distilled water.

STORAGE OF THE KITS

The **Assay Plate, Standard, Detection Reagent A and Detection Reagent B** should be stored at -20°C upon being received. After receiving the kit, **Substrate should be always stored at 4°C**. Other reagents are kept according to the labels on vials. But for long term storage, please keep the whole kit at -20°C (Except the substrate). The unused strips should be kept in a sealed bag with the desiccant provided to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit (six months from the date of manufacture). Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.

Note: Because of the inherent stability of lyophilized material, we may ship these materials at ambient temperature.

SAMPLE COLLECTION AND STORAGE

Serum Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000 × g. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C.

Plasma Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000 × g at 2 - 8°C within 30 minutes of collection. Store samples at -20°C or - 80°C. Avoid repeated freeze-thaw cycles.

Tissue homogenates The preparation of tissue homogenates will vary depending upon tissue type. For this assay, tissue was ice-cold 1x PBS to remove excess blood, homogenized in ice-cold 1x PBS and stored overnight at ≤ -20°C. In most cases, 10% homogenate (eg. 1g of tissue in 10mL of ice-cold 1x PBS) is recommended. After two freeze-thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 5 minutes at 5000 x g. Remove the supernate and assay immediately or aliquot and store at ≤ -20°C.

Cell culture supernates and other biological fluids - Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

Note:

1. Samples to be used within 5 days may be stored at 2-8°C, otherwise samples must be stored at -20°C (1 month) or -80°C (2 months) to avoid loss of bioactivity and contamination.
2. Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts of certain chemicals.
3. Influenced by the factors including cell viability, cell number and also sampling time, samples from cell culture supernatant may not be detected by the kit
4. Sample hemolysis will influence the result, so hemolytic specimen cannot be detected.
5. When performing the assay slowly bring samples to room temperature.

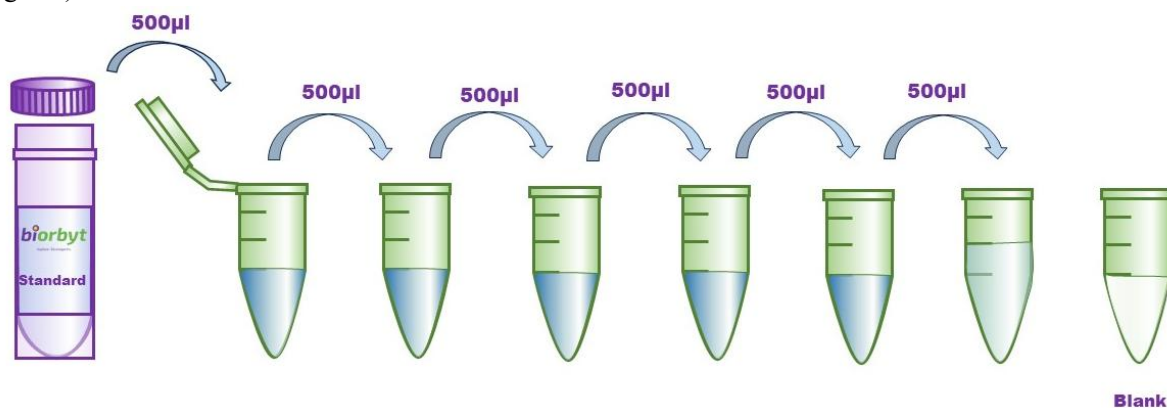
LIMITATIONS OF THE PROCEDURE

1. Biorbyt is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
2. The kit should not be used beyond the expiration date on the kit label.
3. Do not mix or substitute reagents with those from other lots or sources.
4. If samples generate values higher than the highest standard, further dilute the samples with the Sample Diluent and repeat the assay. Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

REAGENT PREPARATION

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 30 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 750 mL of Wash Buffer.

Standard - Reconstitute the Standard with 1.0 mL of Sample Diluent. This reconstitution produces a stock solution of 5000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making serial dilutions (Making serial dilution in the wells directly is not permitted). The undiluted standard serves as the high standard (5000 pg/mL). The Sample Diluent serves as the zero standard (0 pg/mL).



Tube	S7	S6	S5	S4	S3	S2	S1	S0
pg/mL	5000	2500	1250	625	312	156	78	0

Detection Reagent A and B - Dilute to the working concentration using Assay Diluent A and B (1:100), respectively.

ASSAY PROCEDURE

Allow all reagents to reach room temperature (Please do not dissolve the reagents at 37°C directly.). **All the reagents should be mixed thoroughly by gently swirling before pipetting.**

Avoid foaming. Keep appropriate numbers of strips for 1 experiment and remove extra strips from microtiter plate. Removed strips should be resealed and stored at -20°C until the kits expiry date. **Prepare all reagents, working standards and samples as directed in the previous sections.** Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

1. Add 100 μ L of Standard, Blank, or Sample per well. Cover with the Plate sealer. Incubate for 2 hours at 37°C.
2. Remove the liquid of each well, don't wash. Add 100 μ L of Detection Reagent A working solution to each well. Cover with the Plate sealer. Incubate for 1 hour at 37°C. Detection Reagent A working solution may appear cloudy. Warm to room temperature and mix gently until solution appears uniform.
3. Aspirate each well and wash, repeating the process three times for a total of three washes. Wash by filling each well with Wash Buffer (approximately 300 μ L) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. and let it sit for 1~2 minutes. 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 blot it against clean paper towels.
4. Add 100 μ L of Detection Reagent B working solution to each well. Cover with a new Plate sealer. Incubate for 1 hour at 37°C.
5. Repeat the aspiration/wash process for 5 times as conducted in step 3.
6. Add 90 μ L of Substrate Solution to each well. Cover with a new Plate sealer. Incubate within 10-20 minutes at 37°C. Protect from light.
7. Add 50 μ L of Stop Solution to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
8. Determine the optical density of each well at once, using a microplate reader set to 450 nm.

Note:

1. Absorbance is a function of the incubation time. Therefore, prior to starting the assay it is recommended that all reagents should be freshly prepared prior to use and all required strip- wells are secured in the microtiter frame. This will ensure equal elapsed time for each pipetting step, without interruption.
2. Please carefully reconstitute Standards or working Detection Reagent A and B according to the instruction, and avoid foaming and mix gently until the crystals have completely dissolved. **The reconstituted Standards, Detection Reagent A and B can be used only once.** This assay requires

pipetting of small volumes. To minimize imprecision caused by pipetting, ensure that pipettors are calibrated. It is recommended to suck more than 10 μ L for once pipetting.

3. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, DO NOT let the strips DRY at any time during the assay.
4. For each step in the procedure, total dispensing time for addition of reagents to the assay plate should not exceed 10 minutes.
5. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
6. The wash procedure is critical. insufficient washing will result in poor precision and falsely elevated absorbance readings.
7. Duplication of all standards and specimens, although not required, is recommended.
8. Substrate Solution is easily contaminated. Please protect it from light.
9. The web version of manual is only for reference, subject to the instruction shipping with the kit.

SPECIFICITY

This assay recognizes recombinant and natural Human Orexin receptor type 1, Ox1R. No significant cross-reactivity or interference was observed.

Note:

Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between general target antigen and all the analogues, therefore, cross reaction may still exist.

DETECTION RANGE

78.0 pg/mL -5000 pg/mL.

SENSITIVITY

less than 34 pg/mL

PERFORMANCE CHARACTERISTICS

Intra-Assay CV: $\leq 4.7\%$

Inter-Assay CV: $\leq 7.5\%$

Spike Average Recovery: 94%

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample, then subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the x-axis against the concentration on the y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the target antibody concentrations versus the log of the O.D, and the best fit line can be determined by

regression analysis. It is recommended to use some professional software to do this calculation, such as CurveExpert. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

IMPORTANT NOTE

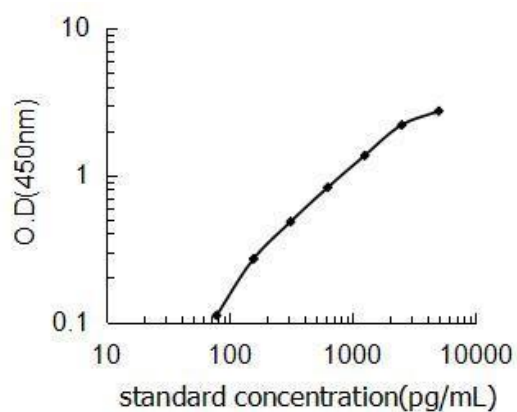
1. Please carefully reconstitute Standards or working Detection Reagent A and B according to the instruction, and avoid foaming and mix gently until the crystals have completely dissolved. The reconstituted Standards, Detection Reagent A and B can be used only once.
2. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, DO NOT let the strips DRY at any time during the assay.
3. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
4. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
5. Substrate Solution is easily contaminated. Please protect it from light.
6. Kits from different batches may be a little different in detection range, sensitivity and color developing time. Please perform the experiment exactly according to printed instruction inside in the kit while electronic ones from our website are for reference only.
7. Do not substitute reagents from one lot to another. Use only the reagents in the same kit supplied by manufacturer.
8. Even the same operator might get different results in two separate experiments. In order to get better reproducible results, the operation of every step in the assay should be controlled. Furthermore, a preliminary experiment before assay for each batch is recommended.
9. Each kit has been strictly passed Q.C test. However, results from end users might be inconsistent with our in-house data due to some unexpected transportation conditions or different lab equipment. Intra-assay variance among kits from different batches might arise from above factors, too.
10. Kits from different manufacturers for the same item might produce different results, since we haven't compared our products with other manufacturers.
11. The instruction manual also suit for the kit of 48T, but all reagents of 48T kit is reduced by half.

PRECAUTION

The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

TYPICAL DATA

This graph data is shown as an example. A standard curve must be generated each time the assay is run.



pg/ mL	Mean O.D.
5000	2.715
2500	2.183
1250	1.355
625	0.822
312	0.481
156	0.269
78	0.111
0	0