ABIN6963403

Competition ELISA Kit for Pregnenolone

Various Species

96 tests

For research use only
Not for use in clinical diagnostic procedures
Version Aug 2024

Intended use

The kit is a competitive inhibition enzyme immunoassay technique for the in vitro quantitative measurement in various sample types.

Reagents and materials provided

- Pre-coated, ready to use 96-well strip plate, flat buttom
- Plate sealer for 96 wells
- Reference Standard
- Reference Standard & Sample Diluent
- Biotinylated Detection Antibody (100 x concentrate)
- HRP Conjugate (100 x concentrate)
- Biotinylated Detection Antibody Diluent
- HRP Conjugate Diluent
- Substrate Reagent
- Stop Solution
- Wash Buffer (25 x concentrate)
- Instruction manual

Materials required but not supplied

1. Microplate reader with 450 ± 10nm filter.
2. Precision single or multi-channel pipettes and disposable tips.
3. Microcentrifuge tubes for diluting samples.
4. Deionized or distilled water.
5. Absorbent paper for blotting the microtiter plate.
6. Container for Wash Solution
7. Incubator capable of maintaining 37 °C.

Storage of the kit

1. For unopened kit: All reagents should be stored according to the labels on the vials, so they are stable up to 12 months after receipt of the kit. The reference standard, biotinylated detection antibody, HRP conjugate, and 96-well strip plate should be stored at -20 °C upon receipt, while the other reagents should be stored at 4 °C.
2. For used kits: When the kit is used, the remaining reagents must be stored according to the above storage conditions. In addition, please return the unused wells to the foil pouch containing the desiccant and seal the foil pouch with the zipper.

.

Note:

It is strongly recommended to use the remaining reagents within 1 month, if this is done before the expiry date of the kit. Please refer to the label on the kit packaging for the expiration date of the kit. All components are stable until the expiration date.

Sample collection and storage

Sample type	Collection procedure
Cell Culture Supernatant	Centrifuge samples for 20 min at 1000×g at 2-8 °C. Collect the supernatant and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles
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 and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles. Hemolysed samples are not suitable for ELISA assay!
Serum	Use a serum separator tube and allow samples to clot for 2 hours at room temperature or overnight at 4°C before centrifugation for 20 min at 1000×g. Collect the supernatant and assay freshly prepared serum immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

Note:

1. Samples to be used within 5 days may be stored at 4 °C, otherwise samples must be stored at -20 °C (≤ 1 month) or -80 °C (≤ 2 months) to avoid loss of bioactivity and contamination.
2. Sample hemolysis will influence the result, so hemolytic specimen should not be used.

3. When performing the assay, bring samples to room temperature.

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 10,000xg 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 50 ng/mL. Then make serial dilutions as needed. The recommended dilution gradient is as follows: 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78, 0 ng/mL. Dilution method: Take 7 EP tubes, add 500 µLof Reference Standard & Sample Diluent to each tube. Pipette 500 µLof the 50 ng/mL stock solution to the first tube and mix up to produce a 25 ng/mL working solution. Pipette 500 µLof 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. Biotinylated Detection Antibody working solution: Calculate the required amount before the experiment (50 µL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the stock tube before use, dilute the 100x Concentrated Biotinylated Detection Antibody to 1xworking solution with Biotinylated Detection Antibody Diluent.
5. Concentrated HRP Conjugate working solution: Calculate the required amount before the experiment (100 µL/well). In preparation, slightly more than calculated should be prepared. Dilute the 100x Concentrated HRP Conjugate to 1x working solution with Concentrated HRP Conjugate Diluent.

Sample preparation

- It is recommended to use fresh samples without long storage, otherwise protein degradation and denaturation may occur in these samples, leading to false results. Samples should therefore be stored for a short period at 2 - 8 °C or aliquoted at -20 °C (≤1 month) or -80 °C (≤ 3 months). Repeated freeze-thaw cycles should be avoided. Prior to assay, the frozen samples should be slowly thawed and centrifuged to remove precipitates.

- If the sample type is not specified in the instructions, a preliminary test is necessary to determine compatibility with the kit.
- 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. The recommended dilution factor is for reference only.
- Please estimate the concentration of the samples before performing the test. If the values are not in the range of the standard curve, the optimal sample dilution for the particular experiment has to be determined.

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 (50 µL for each well). Add the samples to the other wells (50 µL for each well). Immediately add 50 µL of Biotinylated Detection Antibody Working Solution to each well. Cover the plate with the sealer provided in the kit. Incubate for 45 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. Aspirate or decant the solution from each well, add 350 µL 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.
3. Add 100 µL of HRP Conjugate Working Solution to each well. Cover with the Plate Sealer. Incubate for 30 min at 37 °C.
4. Aspirate or decant the solution from each well, repeat the wash process for five times as conducted in step 2.
5. Add 90 µL of Substrate Reagent to each well. Cover with a new Plate Sealer. Incubate for about 15 min 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 30 min.
6. 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.
7. Determine the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm.

Test principle

This ELISA kit uses the Competitive ELISA principle. The micro ELISA plate included in this kit has been pre-coated with the target. During the reaction, the target in the sample or standard competes with a fixed amount of the target on the solid phase support for the target specific sites on the Biotinylated Detection Antibody. Excess conjugate and unbound sample or standard are washed from the plate, and horseradish peroxidase (HRP)-conjugated avidin is added to each well of the microplate and incubated. Then a TMB Substrate Solution is added to each well. The enzyme-substrate reaction is terminated by the addition of Stop Solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of the target in the samples is then determined by comparing the OD of the samples to the standard curve.

Calculation of results

Average the duplicate readings for each standard and sample. Plot a four-parameter logistic curve on log-log graph paper with the standard concentration on the x-axis and the OD values on the y-axis. If the samples were diluted, multiply the concentration calculated from the standard curve by the dilution factor. If the OD of the sample is below the lowest limit of the standard curve, you should retest it with an appropriate dilution. The actual concentration is the calculated concentration multiplied by the dilution factor.

Typical data

In order to make the calculation easier, we plot the O.D. value of the standard (X-axis) against the log of concentration of the standard (Y-axis), although concentration is the independent variable and O.D. value is the dependent variable. The O.D. values of the standard curve may vary according to the conditions of assay performance (e.g. operator, pipetting technique, washing technique or temperature effects).

Detection range

The detection range of the kit is 0.78 ng/mL - 50 ng/mL
The standard curve concentrations used for the ELISA's were 50 ng/mL, 25 ng/mL, 12.5 ng/mL, 6.25 ng/mL, 3.13 ng/mL, 1.56 ng/mL, 0.78 ng/mL, 0 ng/mL

Sensitivity

The minimum detectable dose of target is typically less than 0.47 ng/mL.
The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest protein concentration that could be differentiated from zero.
It was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

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

Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, mid range and high level P5 were tested 20 times on one plate, respectively.
Inter-assay Precision (Precision between assays): 3 samples with low, mid range and high level P5 were tested on 3 different plates, 20 replicates in each plate.
Both intra-CV and inter-CV are < 10 %.

Stability

The stability of ELISA kit is determined by the loss rate of activity. The loss rate of this kit is less than 5 % prior to the expiration date under appropriate storage condition. To minimize extra influence on the performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly monitored. It is also strongly suggested that the assay is performed by the same operator from the beginning to the end.

Assay procedure summary

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

Important note

1. The kit is designed for research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.
2. Limited by the current conditions and scientific technology, we cannot perform a

complete identification and analysis of the raw material used. Therefore, the use of the kit may be associated with some qualitative and technical risks.

3. We are only responsible for the kit itself, not for the samples used in the test. The possible amount of sample used in the whole test should be calculated in advance and sufficient sample material should be provided.
4. Each kit undergoes a very strict QC testing. Nevertheless, end-user results may differ from our internal results due to unexpected transport conditions or different laboratory equipment. Intra-assay deviations between kits from different lots can also be related to this.
5. The test results depend on the validity of the products, so the kit should be used before the expiration date and stored according to the instructions.
6. Even the same user may obtain different results in two separate experiments. To obtain reproducible results, each step of the assay should be controlled.
7. The standard of the kit and immunogen used for antibody preparation are commonly recombinant proteins, as different fragments, expression systems, purification methods might be used in recombinant protein preparation, we can not guarantee the kit could detect recombinant protein from other companies. So, it is not recommended to use the kit for the detection of recombinant protein.
8. Do not mix or substitute reagents from one kit lot to another. Use only the reagents supplied by the manufacturer.
9. Protect all reagents from strong light during storage and incubation. All reagent bottle caps should be tightly closed to prevent evaporation and contamination with microorganisms. The TMB substrate should remain colourless until it reacts with the enzyme.
10. A freshly opened ELISA plate may show a water-like substance, which is normal and does not affect the test results. Return unused wells to the foil pouch and store as before.
11. Mistakes in reagent preparation and application, as well as incorrect parameter setting for the plate reader, can lead to incorrect results. A microplate reader with a bandwidth of 10nm or less and an optical density range of 0-3 O.D. at a wavelength of 450 ± 10 nm is suitable for absorbance measurement. Please read the instructions carefully and set up the instrument before the experiment.
12. Do not reuse the reconstituted standard and the prepared working solutions. The unused stock solutions should be stored according to the storage conditions.

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.

Troubleshooting

Problem	Possible Source	Corrective Action
Poor Standard Curve	Improper standard curve preparation	Ensure accurate operation of the dilution
	Incomplete washing and aspiration	Adequate washing and adequate aspiration
	Inaccurate Pipetting	Check and Calibrate pipettes
Poor Precision	Incomplete washing of wells	Ensure sufficient washing
	Inadequate mixing and aspiration reagents	Adequate aspiration and mixing reagents
	Reused pipette tips, containers and sealers	Change and use new pipette tips, containers and sealers
	Inaccurate Pipetting	Check and Calibrate pipettes
Low O.D Values	Inadequate reagent volumes added to wells	Calibrate pipettes and add adequate reagents
	Incorrect incubation times	Ensure sufficient incubation times
	Incorrect incubation temperature	Reagents balanced to room temperature
	Conjugate or substrate reagent failure	Mix conjugate and substrate, color should develop immediately
	No stop solution added	Follow the assay protocol in the kit manual
	Read beyond suggested reading time	Read within the time recommended in the manual
Sample Values	Improper Sample Storage	Store the sample properly and use the fresh sample

Problem	Possible Source	Corrective Action
	Improper sample collection and preparation	Take proper sample collection and preparation method
	Low quantity of analyte in samples	Resample and repeat assay

For more information, please contact:

antibodies-online Inc.
PO Box 5201
Limerick, PA 19468
USA

Website: www.antibodies-online.com
Email: info@antibodies-online.com
Phone: +1 877 302 8632
Fax: +1 888 205 9894

antibodies-online GmbH
Schloss-Rahe-Straße 15
52072 Aachen
Deutschland

Website: www.antikoerper-online.de
Email: info@antikoerper-online.de
Phone: +49 (0)241 95 163 153
Fax: +49 (0)241 95 163 155