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ABIN6955081

Competition ELISA Kit for Corticotropin Releasing Hormone

Human

96 tests

For research use only
Not for use in clinical diagnostic procedures
Version Mar 2023

Intended use

The kit is a competitive inhibition enzyme immunoassay technique for the in vitro quantitative measurement of CRH in human serum, plasma.

Reagents and materials provided

- Pre-coated, ready to use 96-well strip plate, flat buttom
- Plate sealer for 96 wells
- Reference Standard
- Standard Diluent
- Detection Reagent A
- Detection Reagent B
- Assay Diluent A
- Assay Diluent B
- Reagent Diluent (if Detection Reagent is lyophilized)
- TMB Substrate
- Stop Solution
- Wash Buffer (30 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.
8. 0.01mol/L (or 1×) Phosphate Buffered Saline (PBS), pH7.0-7.2.

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Storage of the kit

1. For unopened kit: All reagents should be stored according to the labels on the vials. The Standard, Detection Reagent A, Detection Reagent B, 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 opened kits: 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.

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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
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.

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Reagent preparation

1. Bring all kit components and samples to room temperature (18-25 °C) before use. If the kit will not be used up in one time, please only take out strips and reagents for present experiment, and leave the remaining strips and reagents in required condition.
2. Standard - Reconstitute the Standard with 0.5mL of Standard Diluent, kept for 10 minutes at room temperature, shake gently (not to foam). The concentration of the standard in the stock solution is 1,000 pg/mL. Prepare 5 tubes containing 0.6 mL Standard Diluent and produce a triple dilution series. Mix each tube thoroughly before the next transfer. Set up 5 points of diluted standard such as 1,000pg/mL, 333.33pg/mL, 111.11pg/mL, 37.04pg/mL, 12.35pg/mL, and the last tube with Standard Diluent is the blank as 0pg/mL.
3. Detection Reagent A and Detection Reagent B - If lyophilized reconstitute the Detection Reagent A with 150μL of Reagent Diluent, kept for 10 minutes at room temperature, shake gently (not to foam). Briefly spin or centrifuge the stock Detection A and Detection B before use. Dilute them to the working concentration 100-fold with Assay Diluent A and B, respectively.
4. Wash Solution - Dilute 20 mL of Wash Solution concentrate (30x) with 580 mL of deionized or distilled water to prepare 600 mL of Wash Solution (1x).
5. TMB substrate - Aspirate the needed dosage of the solution with sterilized tips and do not dump the residual solution into the vial again.

Note:

1. Making serial dilution in the wells directly is not permitted.
2. Prepare standard within 15 minutes before assay. Please do not dissolve the reagents at 37 °C directly.
3. Detection Reagent A and B are sticky solutions, therefore, slowly pipette them to reduce the volume errors.
4. Please carefully reconstitute Standards or working Detection Reagent A and B according to the instruction, and avoid foaming and mix gently until the crystals are completely dissolved. To minimize imprecision caused by pipetting, use small volumes and ensure that pipettors are calibrated. It is recommended to suck more than 10μL for one pipetting.
5. The reconstituted Standards, Detection Reagent A and Detection Reagent B can be used only once.
6. If crystals have formed in the Wash Solution concentrate (30x), warm to room temperature and mix gently until the crystals are completely dissolved.
7. Contaminated water or container for reagent preparation will influence the detection

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result.

Sample preparation

- It is recommended to use fresh samples without long storage, otherwise protein degradation and denaturationmay occur in these samples, leading to false results. Samples should therefore be stored for a short periodat 2 - 8 °C or aliquoted at -20 °C (≤1 month) or -80 °C (≤ 3 months). Repeated freeze-thawcycles should be avoided. Prior to assay, the frozen samples should be slowly thawed and centrifuged toremove precipitates.
- If the sample type is not specified in the instructions, a preliminary test is necessary to determinecompatibility with the kit.
- If a lysis buffer is used to prepare tissue homogenates or cell culture supernatant, there is a possibilityof 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 therange of the standard curve, the optimal sample dilution for the particular experiment has to be determined.Samples should then be diluted with PBS (pH =7.0-7.2).

Assay procedure

1. Determine wells for diluted standard, blank and sample. Add 50 µL each of standard dilutions (read Reagent Preparation), blank, and sample to the appropriate wells. Immediately add 50 µL of Detection Reagent A to each well. Shake the plate gently (use of a microplate shaker is recommended). Cover the plate with a Plate Sealer. Incubate at 37 °C for 1 hour.
2. Aspirate the solution and add 350 µL 1x Wash Solution into each well using a squirt bottle, multichannel pipette, manifold dispenser, or automated washer and allow to soak for 1-2 minutes. Completely remove the remaining liquid from all wells by tapping the plate on absorbent paper. Repeat the process 3 times. After the last wash, remove all remaining Wash Buffer by aspirating or decanting. Turn the plate over and blot it against absorbent paper.
3. Add 100 µL of Detection Reagent B Working Solution to each well. Incubate for 30 minutes at 37 °C after covering the plate with a Plate Sealer.
4. Repeat the aspiration/washing procedure a total of 5 times as performed in step 2.
5. Add 90 µL of Substrate Solution to each well. Cover with a new Plate Sealer. Incubate for 15 - 25 minutes at 37 °C (no longer than 30 minutes). Protect from light. The liquid

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will turn blue with the addition.

6. Add 50 µL of Stop Solution to each well. The liquid will turn yellow. Mix the liquid by tapping the side of the plate. If the color change does not appear even, gently tap the plate to ensure thorough mixing.
7. Remove any water droplets or fingerprints from the bottom of the plate and make sure there are no bubbles on the surface of the liquid. Then start the microplate reader and immediately take a measurement at 450 nm.

Test principle

This assay employs the competitive inhibition enzyme immunoassay technique. An antibody specific to target has been pre-coated onto a microtiter plate. A competitive inhibition reaction is launched between biotin labeled target and unlabeled target (Standards or samples) with the pre-coated antibody specific to target. After incubation the unbound conjugate is washed off. Next, avidin conjugated to Horseradish Peroxidase (HRP) is added to each microtiter plate well and incubated. The amount of bound HRP conjugate is reverse proportional to the concentration of target in the sample. After addition of the substrate solution, the intensity of color developed is reverse proportional to the concentration of target in the sample.

Calculation of results

This assay uses the competitive inhibition enzyme immunoassay technique, so there is an inverse correlation between the concentration in the sample and the signal intensity. Average the duplicate readings for each standard, control, and samples. Create a standard curve on log-log or semilogarithmic graph paper with the logarithm of the concentration on the y-axis and the absorbance on the x-axis. Draw the best fit line through the points that could be determined by regression analysis. The use of plotting software is also recommended. If the samples have been diluted, the concentration read from the standard curve must be 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).

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Detection range

The detection range of the kit is 12.35 pg/mL - 1000 pg/mL
The standard curve concentrations used for the ELISA's were 1000pg/mL, 333.33pg/mL, 111.11pg/mL, 37.04pg/mL, 12.35pg/mL, 0.00 pg/mL

Sensitivity

The minimum detectable dose of CRH is typically less than 5.19 pg/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 CRH and all the analogues, therefore, cross reaction may still exist.

Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level of target were tested 20 times on one plate, respectively.
Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level of target were tested on 3 different plates, 8 replicates in each plate.
CV(%) = SD/meanX100
Intra-Assay: CV < 10%
Inter-Assay: CV < 12%

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. Prepare all reagents, samples and standards,

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2. Add 50μL standard or sample to each well.
   Then add 50μL prepared Detection Reagent A immediately.
   Shake and mix. Incubate 1 hour at 37 °C,
3. Aspirate and wash 3 times,
4. Add 100μL prepared Detection Reagent B. Incubate 30 minutes at 37 °C,
5. Aspirate and wash 5 times,
6. Add 90μL Substrate Solution. Incubate 10-20 minutes at 37 °C,
7. Add 50μL Stop Solution. Read at 450 nm immediately.

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

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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

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

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