Product Manual

Revision date: 08 Oct 2019

Human Adrenal Cortex Antibody (ACA) ELISA Kit

Catalog No.: abx053206

Range: Qualitative

Sensitivity: Qualitative

Storage: Store at 2-8°C for 6 months.

Application: For qualitative detection of ACA in Human Serum, Plasma, Tissue Homogenates and cell culture supernatants.

Introduction: Adrenal Cortex Antibodies (ACA) are autoantibodies against 21α-hydroxylase (CYP21A2) and/or 17α-hydroxylase (CYP17A1). These enzymes are responsible for the addition of a hydroxyl group to carbon 21 and 17 (respectively) on steroid hormones. ACA are responsible for most cases of Addison's disease in countries where tuberculosis is uncommon; symptoms of this illness include hyperpigmentation and hypotension, and can cause an adrenal crisis if untreated.

Principle of the Assay

A 96 well plate has been pre-coated with the target antigen. Controls or test samples are added to the appropriate wells and incubated. Free components are washed away with wash buffer. HRP conjugated detection reagent is used to visualize HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue colour product that changes to yellow after adding acidic stop solution. The intensity of the color yellow is proportional to the ACA amount bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450 nm in a microplate reader, and the presence of ACA can be determined.

Kit components

1. One pre-coated 96-well microplate (8 × 12 well strips)
2. Positive Control: 0.5 ml
3. Negative Control: 0.5 ml
4. Wash buffer (20X): 25 ml. Dilution: 1:20
5. Sample diluent buffer: 6 ml
6. HRP Conjugate Reagent (RTU): 10 ml
7. Stop solution: 6 ml
8. TMB substrate A: 6 ml
9. TMB substrate B: 6 ml
10. Plate sealer: 2
11. Hermetic bag: 1

Material Required But Not Provided

1. 37°C incubator
2. Microplate reader (wavelength: 450 nm)
3. Multi and single channel pipettes and sterile pipette tips
4. Squirt bottle or automated microplate washer
5. ELISA shaker
6. 1.5 ml tubes
7. Deionized or distilled water
8. Absorbent filter papers
9. 100 ml and 1 liter graduated cylinders

Protocol

A. Preparation of sample and reagents

1. Sample

Isolate the test samples soon after collecting and analyze immediately at 1/5 dilution (within 2 hours) or aliquot and store at -20°C or -80°C for long term storage. Avoid multiple freeze-thaw cycles.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 60 minutes. Centrifuge at approximately 1000 × g for 20 min. Analyze the serum immediately or aliquot and store at -20°C or -80°C.
- **Plasma:** Collect plasma using citrate or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 × g within 30 minutes of collection. Assay immediately or aliquot and store at -20°C. Avoid hemolysis and high cholesterol samples.
- **Tissue homogenates:** The preparation of tissue homogenates will vary depending upon tissue type – this is just an example. Rinse tissues with ice-cold PBS to remove the excess of blood. Weigh before homogenization. Finely mince tissues and homogenize with a tissue homogenizer on ice in PBS and sonicate the cell suspension. Centrifuge the homogenates at 5000 × g for 5 min and collect the supernatant. Assay immediately or aliquot and store at -20°C.
- **Cell culture supernatant:** Centrifuge at approximately 2000-3000 × g for 20 min to remove precipitant. Analyze immediately or aliquot and store at -20°C or -80°C.

Note:

» Coagulate blood samples completely, centrifuge, and avoid hemolysis and precipitant.

» NaN_3 cannot be used as test sample preservative, since it inhibits HRP.

» Store samples undiluted. Once ready to analyze, thaw samples and dilute 1/5.

2. Wash buffer

Dilute the concentrated Wash buffer 20-fold (1/20) with distilled water (i.e. add 20 ml of concentrated wash buffer into 380 ml of distilled water).

B. Assay Procedure

Equilibrate the kit components and samples to room temperature prior to use.

1. Set positive/negative controls, test sample and control (zero/blank) wells on the pre-coated plate respectively and record their positions.
2. Aliquot 50 µl of the negative and positive controls into the set wells. Leave one well as the control (zero) blank well.
3. Aliquot 50 µl of appropriately diluted sample into the test sample wells. Samples should be diluted 1/5. Add the solution at the bottom without touching the side walls of the well. Shake the plate gently to mix the contents.
4. Seal the plate with a cover and incubate at 37°C for 30 min.
5. Remove the cover and discard the solution. Wash the plate 5 times with 1X Wash Buffer. Fill each well completely with Wash buffer (300 µl) using a multi-channel Pipette or autowasher (1-2 minute soaking period is recommended). Complete removal of liquid at each step is essential for good performance. After the final wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean absorbent paper towels.
6. Add 100 µl of HRP conjugate reagent into each well (except the blank well). Add the solution at the bottom of each well without touching the side wall.
7. Seal the plate with a cover and incubate at 37°C for 60 min.
8. Remove the cover and repeat the aspiration/wash process 5 times as explained in step 5.

9. Aliquot 50 µl of TMB Substrate A into each well, and then add 50 µl of TMB Substrate B . Vortex the plate gently on an ELISA shaker for 30 seconds (Or shake gently by hand for 30 seconds). Cover the plate and incubate at 37°C for 15 min. Avoid exposure to light.
10. Add 50 µl of Stop solution into each well to stop the enzyme reaction. It is important that the Stop Solution is mixed quickly and uniformly throughout the microplate to inactivate the enzyme completely.
11. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the absorbance at 450 nm immediately.

C. Analysis

1. **Calculations:**
   Mean absorbance of the positive control should be ≥ 1.00.
   Mean absorbance of the negative control should be ≤ 0.15.
   CUT OFF value = Negative control + 0.15
2. **Interpretation of results:**
   If the postive control value is ≥ 1.00 and the negative control value is ≤ 0.15, the test is valid, otherwise, the test is invalid.
   Samples:
   If O.D. of samples < CUT OFF, the test samples are considered negative.
   If O.D. of samples ≥ CUT OFF, the test samples are considered positive.

D. Precautions

1. Before using the kit, centrifuge the tubes to bring down the contents trapped in the lid.
2. Avoid foaming or bubbles when mixing or reconstituting components.
3. If crystals have formed in the concentrated Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.
4. It is recommended measuring each controls and samples in duplicate.
5. Do not let the wells uncovered for extended periods between incubation. Once reagents are added to the wells do not let the strips dry as this can inactivate the biological material on the plate. Incubation time and temperature must be controlled..
6. Ensure plates are properly sealed or covered during incubation steps.
7. Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings.
8. Do not reuse pipette tips and tubes to avoid cross contamination.
9. Do not use expired components or components from a different kit.
10. The TMB Substrate B is easily contaminated; protect from light and work under sterile conditions when handling the TMB substrate solution. Equilibrate the TMB substrate at room temperature prior to use. Unreacted substrate should be colorless or very light yellow in appearance. Aspirate the dosage needed with sterilized tips and do not dump the residual solution back into the vial.