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Human ACTH ELISA Kit

Catalog number: EK7007

For detection of multiple analytes using one single assay.

This package insert must be read in its entirety before using this product.

For research use only. Not for use in diagnostic procedures.

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Human ACTH ELISA Kit

Catalog Number: EK7007

Size: 96 wells/kit

Sample Type: EDTA plasma

Sensitivity: 6.8 pg/ml

Assay Range: 6.8-531 pg/ml

Storage: Store the kit at 2°C to 8°C. Keep microwells sealed in a dry bag with desiccants. The reagents are stable until expiration of the kit. Do not expose reagent to heat, sun, or strong light. Avoid multiple freeze-thaw cycles (Ships with gel ice, can store for up to 3 days in room temperature. Freeze upon receiving.)

Introduction

The ACTH Immunoassay is a two-site ELISA for the measurement of the biologically active 39 amino acid chain of ACTH. One antibody is prepared to bind only the C-terminal ACTH 34-39 and this antibody is biotinylated. The other antibody is prepared to bind only the mid-region and N-terminal ACTH 1-24 and this antibody is labeled with HRP for detection. In this assay, calibrators, controls, or patient samples are simultaneously incubated with the enzyme labeled antibody and a biotin coupled antibody in a streptavidincoated microplate well. At the end of the assay incubation, the microwell is washed to remove unbound components and the enzyme bound to the solid phase is incubated with the TMB substrate. Stop solution is then added to stop the reaction and converts the color to yellow. The intensity of the yellow color is directly proportional to the concentration of ACTH in the sample. A dose response curve of absorbance unit vs. concentration is generated using results obtained from the calibrators. Concentrations of ACTH present in the controls and patient samples are determined directly from this curve.

Kit Components

Description	Quantity
Microwells coated with Streptavidin	12x8x1 Microwells
Biotinylated ACTH Antibody (Reagent 1)	2.7 ml
Peroxidase (Enzyme) labeled ACTH Antibody	(1 Vial) 2.7 ml
Wash Concentrate	(1 Vial) 30 ml
TMB Substrate	1 vial 15ml
Stop Solution	1 Vial 20 ml
Calibrators	5 Vials 2 ml
Zero Calibrator	(1 Vial) 4 ml
Controls 1 & 2 (CTRL)	(2 Vials) 2 ml

Materials Required, but Not Provided

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips. Multichannel pipettes are recommended in the condition of large amount of samples in the detection.
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

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WARNINGS AND PRECAUTIONS

1. For Research Use Only. Not for use in diagnostic procedures.

2. Potential biohazardous materials: The calibrator and controls contain human source components, which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent. These reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories" 1984.

3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.

4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

5. It is recommended that standards, control and serum samples be run in duplicate.

6. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

7. This product contains components preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.

SPECIMEN COLLECTION HANDLING

1. The determination of ACTH should be performed on EDTA plasma.

2. To assay the specimen in duplicate, 400 ul of EDTA plasma is required.

3. Collect whole blood in a lavender [EDTA] tube.

4. The plasma should be promptly separated, preferably in a refrigerated centrifuge, and stored at -20°C or lower.

5. EDTA plasma samples may be stored up to 8 hours at 2-8°C.

6. EDTA plasma samples frozen at -20°C are stable for up to 4 months.

REAGENT PREPARATION

Store all kit components at 2-8°C except Wash Concentrate and Stop Solution.

All reagents except the non-zero calibrators, kit controls and the Wash Concentrate are ready-to-use. Store all reagents at 2-8°C, except the Wash Concentrate, which should be kept at room temperature until dilution to avoid precipitation.

For each of the non-zero calibrators (Calibrator B through F) and kit controls 1 and 2, reconstitute each vial with 2 ml of distilled or deionized water and mix. Allow the vial to stand for 10 minutes and then mix thoroughly by gentle inversion to insure complete reconstitution. Use the calibrators and controls as soon as possible upon reconstitution. Freeze (-20°C) the remaining calibrators and controls as soon as possible after use. Calibrators and controls are stable at -20°C for 6 weeks after reconstitution with up to 3 freeze thaw cycles when handled as recommended in “Procedural

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Notes" section.

ELISA Reagent A: Wash Concentrate: Mix contents of wash concentrate thoroughly. If precipitate is present in the Wash Concentrate due to storage at lower temperature such as 4°C, dissolve by placing the vial in a 37°C water bath or oven with swirling or stirring. Add wash concentrate (30 ml) to 570 ml of distilled or deionized water and mix. The diluted working wash solution is stable for 90 days when stored at room temperature.

ASSAY PROCEDURE

Prior to assay, allow reagents to stand at room temperature (20-25°C).
Gently mix all reagents before use.

1. Place sufficient Streptavidin Coated Strips in a holder to run all six (6) ACTH calibrators, A - F of the ACTH CALIBRATORS (concentration is stated on the vial label), Quality Control Plasma and patient samples.

2. Pipet 200 ul of sample into the designated or mapped well. Freeze (-20°C) the remaining calibrators and controls as soon as possible after use.

3. Add or dispense 25 ul of Reagent 1 (Biotinylated Antibody) into each of the wells which already contain the sample.

4. Add or dispense 25 ul of Reagent 2 (Enzyme Labeled Antibody) into each of the same wells. Cover the microplate (s) with aluminum foil or a tray to avoid exposure to light, and place it on an orbital shaker or rotator set at 170 + 10 rpm for 4 hours + 30 minutes at room temperature (20-25°C).

5. First aspirate the fluid completely and then wash/aspirate each well five (5) times with the Working Wash Solution (prepared from Reagent A), using an automatic microplate washer. The wash solution volume should be set to dispense 0.35 ml into each well.

6. Add or dispense 150 uL of the ELISA Reagent B (TMB Substrate) into each of the wells.

7. With appropriate cover to avoid light exposure, place the microplate (s) on an orbital shaker or rotator set at 170 + 10 rpm for 30 +5 minutes at room temperature (20-25°C).

8. Add or dispense 100 ul of the Stopping Solution into each of the wells. Mix gently.

9. Read the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to 450 nm against 250 ul of distilled or deionized water. Read the plate again with the reader set to 405 nm against distilled or deionized water. Note: The second reading is designed to extend the analytical validity of the calibration curve to the value represented by the highest calibrator, which is approximately 500 pg/ml. Hence, patient samples with ACTH > 150 pg/ml can be quantified against a calibration curve consisting of the readings all the way up to the concentration equivalent to the highest calibrator using the 405 nm reading, away from the wavelength of maximum absorbance. In general, patient and control samples should be read using the 450 nm for ACTH concentrations up to 150 pg/ml. ACTH concentrations above 150 pg/ml should be interpolated using the 405 nm reading.

10. By using the final absorbance values obtained in the previous step, construct a calibration curve via cubic spline, 4 parameter logistics, or point-to-point interpolation to quantify the concentration of the ACTH.

CALCULATION OF RESULTS

The standard curve is constructed as follows:

1. For the 450 nm readings, construct a dose response curve (calibration curve) using the first five calibrators provided, i.e. Calibrators A, B, C, D and E. For the 405 nm readings, construct a second dose response curve using the three calibrators with the highest concentrations, i.e. Calibrators D, E

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and F.

2. Assign the concentration for each calibrator stated on the vial in pg/ml. Plot the data from the calibration curve on linear graph paper with the concentration on the X-axis and the corresponding A.U. on the Y-axis.

3. Draw a straight line between 2 adjacent points. This mathematical algorithm is commonly known as the "point-to-point" calculation. Obtain the concentration of the sample by locating the absorbance unit on the Y-axis and finding the corresponding concentration value on the X-axis. Patient and control samples should be read using the 450 nm for ACTH concentrations up to 150 pg/ml. ACTH concentrations above 150 pg/ml should be interpolated using the 405 nm reading.

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