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Product Information

Adrenocorticotropic Hormone (ACTH) ELISA

Catalog Number **SE120140**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Adrenocorticotropic hormone (ACTH) is a 39 amino acid peptide hormone (molecular mass = 4,500 Da) secreted by the pituitary to regulate the production of steroid hormones by the adrenal cortex. ACTH increases the synthesis and release of all adrenal sterioids, aldosterone, cortisol, and adrenal androgens. It is the principal modulator of cortisol, the most important glucocorticoid in man. As the cortisol level in the blood increases, release of ACTH is inhibited directly at the pituitary level. Through this same mechanism, decreasing cortisol levels lead to elevated ACTH levels. In healthy individuals, ACTH reaches a peak in the early morning (6:00 – 8:00 am) and levels become lowest late in the day and near the beginning of the sleep period. Stress may also override the diurnal variation. Plasma ACTH assays are useful in the differential diagnosis of pituitary Cushing's disease, Addison's disease, autonomous ACTH producing pituitary tumors (*e.g.*, Nelson's syndrome), hypopituitarism with ACTH deficiency, and ectopic ACTH syndrome. Hypopituitarism with ACTH deficiency, which is a secondary adrenocortical insufficiency, is characterized by low plasma ACTH and cortisol concentrations, and a subnormal, but usually distinct adrenal response to stimulation with synthetic ACTH (Cortrosyn).

The Adrenocorticotropic Hormone (ACTH) ELISA is intended for the quantitative determination of ACTH in human plasma. The ACTH Immunoassay is a two-site ELISA (Enzyme-Linked ImmunoSorbent Assay) for the measurement of the biologically active 39 amino acid chain of ACTH. A goat polyclonal antibody to ACTH, purified by affinity chromatography, and a mouse monoclonal antibody to ACTH are specific for well defined regions on the ACTH molecule. 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 horseradish peroxidase [HRP] for detection.

In this assay, calibrators, controls, or samples are simultaneously incubated with the enzyme labeled antibody and a biotin coupled antibody in a streptavidin-coated 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 substrate, tetramethylbenzidine (TMB). An acidic Stop Solution is then added to stop the reaction and convert the color to yellow. The intensity of the color is directly proportional to the concentration of ACTH in the sample. A dose response curve of absorbance units versus 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.

Components

Materials Provided	96 Tests
Microwells coated with Streptavidin	12 x 8 x 1
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)	15 mL
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

Reagents and Equipment Required but Not Provided.

- Distilled or deionized water
- Precision pipettes
- Disposable pipette tips
- Microplate luminometer
- Absorbent paper or paper towel
- Graph paper

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Sample Preparation

1. The determination of ACTH should be performed on EDTA plasma.
2. To assay the specimen in duplicate, 400 µL 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.

All reagents except the non-zero calibrators, kit controls, and the Wash Concentrate are ready to use.

Preparation of non-zero Standards/Calibrators

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 ensure 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 Procedure.

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(18–26 °C).

Storage/Stability

Store all reagents at 2–8 °C, except the Wash Concentrate, which should be kept at room temperature until dilution to avoid precipitation.

Procedure

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

Control plasma or plasma pools should be analyzed with each run of calibrators and samples. Results generated from the analysis of the control samples should be evaluated for acceptability using appropriate statistical methods. In assays in which one or more of the quality control sample values lie outside the acceptable limits, the results for the sample may not be valid.

1. Bring all specimens and kit reagents to room temperature (18–26 °C) and mix gently.
2. Place sufficient Streptavidin Coated Strips in a holder to run all six ACTH Calibrators (A–F of the ACTH Calibrators, concentration are stated on the vial labels), Quality Control Plasma, and samples.
3. Pipette 200 µL of sample into the designated or mapped well. Freeze (–20 °C) the remaining calibrators and controls as soon as possible after use.
4. Add or dispense 25 µL of Reagent 1 (Biotinylated Antibody) into each of the wells which already contain the sample.
5. Add or dispense 25 µL of Reagent 2 (Enzyme Labeled Antibody) into each of the 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 (18–26 °C).
6. First aspirate the fluid completely and then wash/aspirate each well five (5) times with the Working Wash Solution using an automatic microplate washer. Set the automatic washer to dispense 0.35 mL of Working Wash Solution into each well.
7. Add or dispense 150 µL of the TMB Substrate into each of the wells.
8. 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 (18–26 °C).
9. Add or dispense 100 µL of the Stop Solution into each of the wells. Mix gently.

10. Read the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to 450 nm against 250 µL 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 ~500 pg/mL. Hence, 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.
11. 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.

Results

Calculations

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, 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 absorbance 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 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.

Note:The ACTH ELISA has exhibited no “high dose hook effect” with samples spiked with 20,000 pg/mL of ACTH. However, samples with ACTH levels greater than the highest calibrator should be diluted and reassayed for correct values. Like any analyte used as a diagnostic adjunct, ACTH results must be interpreted carefully with the overall clinical presentations and other supportive diagnostic tests.

Product Profile

ACTH levels were measured in eighty three (83) apparently normal individuals. The values obtained ranged from 7.9 to 66.1 pg/mL. The geometric mean plus 2 standard deviations of the mean were calculated to be 8.3 to 57.8 pg/mL. It’s recommended that each lab establishes its own normal range.

References

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