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Adrenocorticotropic Hormone [ACTH] (human) ELISA Kit

(Catalog # K4003-100, 100 assays; Store at 2-8^{0}C)

I. Introduction:

ACTH is a 39-amino acid peptide hormone (MW=4500) 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 blood increases, release of ACTH is inhibited directly at the pituitary level. Through this same mechanism, decreasing cortisol levels lead to elevated ACTH levels. 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. BioVision 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 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 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 sample is determined directly from this curve.

II. Application:

Quantitative protein detection

III. Specificity:

Human ACTH.

IV. Sample Type:

- Plasma

V. Kit Contents:

Components	K4003-100	Part No.
Plate coated with Streptavidin	12 stripsx8 wells	K4003-100-1
Biotinylated ACTH Ab (reagent 1)	2.7 ml	K4003-100-2
Peroxidase labeled ACTH Ab	2.7 ml	K4003-100-3
Calibrators	2 ml	K4003-100-4
Zero Calibrator	4 ml	K4003-100-5
Controls 1 & 2 (CTRL)	2 ml	K4003-100-6
ACTH Enzyme Conjugate	12 ml	K4003-100-7
Wash Concentrate	30 ml	K4003-100-8
TMB Substrate	15 ml	K4003-100-9
Stop Solution	20 ml	K4003-100-10

VI. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm.
- Absorbent paper.
- Adjustable pipettes and pipette tips.

VII. Storage Conditions and Reagent Preparation:

- Store kit at 2-8^{o}C except wash concentrate and stop solution. Wash concentrate should be kept at room temperature until dilution to avoid precipitation. Keep microwells sealed in a dry bag with desiccants. Spin tubes briefly to bring down all components to the bottom of tubes. Reagents are stable until the expiration of the kit. Do not expose reagent to heat, sun, or strong light.
- 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 min. 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.
- 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.

VIII. Warning & Precautions:

- Potential biohazardous materials: The Calibrators 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.
- Do not pipette by mouth.
- The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
- 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.
- 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.

IX. Sample Preparation and Storage:

The determination of ACTH should be performed on EDTA plasma. To assay the specimen in duplicate, 400 µl of EDTA plasma is required. Collect whole blood and separate plasma promptly, preferably in a refrigerated centrifuge, and stored at -20°C or lower. EDTA plasma samples may be stored up to 8 hrs at 2-8°C. EDTA plasma samples frozen at -20°C are stable for up to 4 months.

X. Assay Protocol:

Prior to assay, allow reagents to stand at room temperature. Gently mix all reagents before use.

1. Place sufficient number of coated strips into the holder to run all 6 ACTH calibrators (A-F), Quality Control plasma and samples.
2. Pipet 200 µl of sample, calibrators and controls into designated wells.
3. Add 25 µl of Biotinylated Ab into each well. Then add 25 µl of peroxidase Labeled Ab into each well. Cover the microplate(s) with aluminum foil to avoid exposure to light, and gently shake for 4 hrs and 30 min. at room temperature (18-26°C).
4. Aspirate the fluid completely and then wash/aspirate each well five times with 350 µl of Working Wash Solution using an automatic microplate washer.
5. Add 150 µl of TMB Substrate into each well. Cover the plate to protect from light, gently shake for 30-35 min. at room temperature (18-26°C).
6. Add 100 µl of Stop Solution into each well. Mix gently.
7. Read the absorbance within 10 min., 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 approximately 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, 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.
8. By using the final absorbance value, construct a calibration curve via cubic spline, 4 parameter logistics, or point-to-point interpolation to quantify the concentration of the ACTH.

XI. CALCULATION OF RESULTS:

For 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. 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 Yaxis. 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. Samples and controls 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.

XII. QUALITY CONTROL:

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.

XIII. LIMITATIONS OF THE PROCEDURE:

BioVision ACTH ELISA kit has exhibited no “high dose hook effect” with samples spiked with 20,000 pg/ml of ACTH. Samples with ACTH levels greater than the highest calibrator, however, 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.

XIV. EXPECTED VALUES

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 + 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.

XV. RELATED PRODUCTS:

ACTH (4003) Adrenocorticotropic Hormone [ACTH] (mouse/rat) ELISA Kit (K4004)

FOR RESEARCH USE ONLY! Not to be used on humans.