Datasheet for ABIN1305175

ACTH ELISA Kit

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Overview

Quantity:	96 tests
Target:	ACTH
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	1.0-416 pg/mL
Minimum Detection Limit:	1 pg/mL
Application:	ELISA

Product Details

Purpose:	This test kit is intended for use in the quantitative determination of human adrenocorticotropic hormone (ACTH) in EDTA-plasma. The test is useful for detecting elevated and deficient ACTH levels. Indications for use: Patient may have a higher than normal levels of ACTH with1. Addison's disease or primary adrenal insufficiency2. Congenital adrenal hyperplasia3. Cushing's syndrome4. Cushing's disease5. Multiple endocrine neoplasia (MEN), type I Patient may have a lower than normal levels of ACTH with6. Hypopituitarism and/or secondary adrenal insufficiency7. Adrenal gland tumor8. Other tumors that produce cortisol
Brand:	ED™
Sample Type:	Plasma
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Components:	1. Anti-ACTH Antibody Coated Microplate

Product Details

	One vial containing 5 mL ready-to-use buffer. It should be used only for tracer antibody dilution according to the assay procedures. This reagent should be stored at 2-8 °C and is stable until the expiration date on the kit box.
Material not included:	1. Precision single channel pipettes capable of delivering 25 µL, 200 µL, etc. 2. Disposable pipette tips suitable for above volume dispensing. 3. Aluminum foil. 4. Deionized or distilled water. 5. Plastic microtiter well cover or polyethylene film. 6. ELISA multichannel wash bottle or automatic (semi-automatic) washing system. 7. Spectrophotometric microplate reader capable of reading absorbance at 450/650 or 450/620 nm.

Target Details

Target:	ACTH
Abstract:	ACTH Products
Target Type:	Hormone
Background:	ACTH is a 39 amino acid polypeptide with a molecular weight of 4540 Dalton. ACTH is secreted from corticotropes in the anterior lobe (or adenohypophysis) of the pituitary gland in response to corticotropin-releasing hormone (CRH) released by the hypothalamus. ACTH is synthesized from pre-pro-opiomelanocortin (pre-POMC). The removal of the signal peptide during translation produces the 241-amino acid polypeptide POMC, which undergoes a series of post-translational modifications such as phosphorylation and glycosylation before it is proteolytically cleaved by endopeptidases to yield various polypeptide fragments with varying physiological activity. ACTH is an important component of the hypothalamic-pituitary-adrenal axis and is often produced in response to biological stress. It stimulates secretion of glucocorticoid steroid hormones from adrenal cortex cells especially in the zona fasciculata of the adrenal. ACTH acts by binding to cell surface ACTH receptors, which are located primarily on adrenocortical cells of the adrenal cortex.
Pathways:	Metabolism of Steroid Hormones and Vitamin D, Peptide Hormone Metabolism, Hormone Activity

Application Details

Sample Volume:	0.4 mL

Application Details

Assay Time:	4 h
Plate:	Pre-coated
Protocol:	This ELISA kit is designed, developed and produced for the quantitative measurement of human ACTH in EDTA-plasma sample. The assay utilizes the two-site sandwich technique with selected antibodies that bind to N-terminal and C-terminal epitopes of ACTH.Assay standards, controls and patient samples are added directly to wells of a microtiter plate that is coated with antibody to the C-terminal of human ACTH. Immediately, a horseradish peroxidase (HRP) conjugated anti N-terminal of human ACTH antibody is added to each well. After the first incubation period, a sandwich of solid-phase polyclonal antibody - human ACTH HRP conjugated monoclonal antibody is formed. The unbound antibodies and buffer matrix are removed in the subsequent washing step. For the detection of this immunocomplex, the well is then incubated with a substrate solution in a timed reaction, which is terminated with an acidic reagent (i.e. ELISA stop solution). The absorbance is then measured in a spectrophotometric microplate reader. The enzymatic activity of the immunocomplex bound to the wall of each microtiter well is directly proportional to the amount of human ACTH in the test sample. A standard curve is generated by plotting the absorbance versus the respective human ACTH concentration for each standard on a point-to-point or 4-parameter curve fitting. The concentration of human ACTH in test samples is determined directly from this standard curve.
Reagent Preparation:	(1) Prior to use allow all reagents to come to room temperature. Reagents from different kit lot numbers should not be combined or interchanged. (2) ELISA Wash Concentrate must be diluted to working solution prior to use. Please see REAGENTS section for details. (3) Reconstitute assay standards and controls by adding 2.0 mL of deminerialized water to each standard and control bottle. Allow the standard and controls to sit undisturbed for 5 minutes, and then mix well by inversions or gentle vortexing. One must make sure that all solid is dissolved completely prior to use. These reconstituted standards and controls may be stored at 2- 8 C for up to 24 hours or below ?10 C for long-term storage. Do not exceed 3 freeze-thaw cycles. (4) Prepare Tracer Antibody working solution by 1:21 fold dilution of the ACTH Tracer Antibody by adding the tracer antibody into the Tracer Antibody Diluent . Following is a table that outlines the relationship of strips used and antibody mixture prepared. NOTE: the tracer antibody should be prepared just prior to the beginning of the assay. (5) Test Configuration (6) Place a sufficient number of Anti-ACTH antibody-coated microwell strips in a holder to run human ACTH standards, controls and unknown samples in duplicates.

Application Details

Sample Collection:	Since the circulating ACTH shows a 24 hours circadian rhythms, it is recommended to draw blood sample in the early morning, before 8 a.m. Patients should stop taking steroid drugs before drawing blood sample, at the consultation of their physician. EDTA-plasma is a suitable specimen for human ACTH measurement. A total of 0.4 mL EDTA-plasma is required for duplicate determination of human ACTH with this test kit. Whole blood should be collected using lavender-top Vaccutainer and the plasma separated according to manufacturer?s instruction. The EDTA-plasma should be separated from other cells right after or within one hour of blood collection. The plasma should be transferred to a clean test tube right after centrifugation. Plasma samples should be stored at ? 20 °C if the assay is not to be performed within 3 hours. Avoid more than three times freeze-thaw cycles of specimen. Samples of serum, heparin plasma and citrate plasma should not be used for ACTH measurement.
Assay Procedure:	(1) Add 200 µL of standards, controls and patient samples into the designated microwells. (2) Immediately add 25 µL of HRP Conjugated Anti-ACTH Tracer Antibody mix to each well. (3) Seal the plate wells securely, cover with foil or other material to protect from light, and rotate on an ELISA plate shaker (small orbit radius) for 2 hr. 5 minutes at 400 to 450 rpm. (4) Wash each well 5 times by dispensing 350 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used. (5) Add 200 µL of ELISA HRP Substrate into each of the wells. (6) Cover the plate with aluminum foil or other material to avoid exposure to light. Incubate plate static, at room temperature for 20 minutes. (7) Immediately add 50 µL of ELISA Stop Solution into each of the wells. Mix gently. (8) Read the absorbance at 450 nm with reference filter at 620 nm or 650 nm.
Calculation of Results:	It is recommended to use a point-to-point or 4-parameter standard curve fitting. 1. Calculate the average absorbance for each pair of duplicate test results. 2. Subtract the average absorbance of the level 1 standard (0 pg/mL) from the average absorbance of all other readings to obtain corrected absorbance. 3. The standard curve is generated by the corrected absorbance of all standard levels on the ordinate against the standard concentration on the abscissa using point-to-point or log-log paper. Appropriate computer assisted data reduction programs may also be used for the calculation of results. The human ACTH concentrations for the controls and the patient samples are read directly from the standard curve using their respective corrected absorbance.
Assay Precision:	The intra-assay precision was validated by measuring three control samples with 16 replicate determinations. The inter-assay precision was validated by measuring two control levels in duplicate in 16 individual assays.

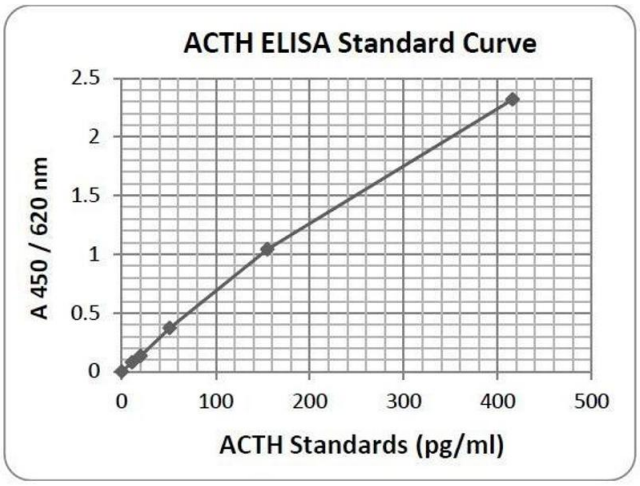
Application Details

Restrictions:	For Research Use only

Handling

Precaution of Use:	The reagents must be used in professional laboratory. Source material for reagents containing bovine serum was derived in the contiguous 48 United States. It was obtained only from healthy donor animals maintained under veterinary supervision and found free of contagious diseases. Wear gloves while performing this assay and handle these reagents as if they are potentially infectious. Avoid contact with reagents containing TMB, hydrogen peroxide, or sulfuric acid. TMB may cause irritation to skin and mucous membranes and cause an allergic skin reaction. TMB is a suspected carcinogen. Sulfuric acid may cause severe irritation on contact with skin. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes. Use Good Laboratory Practices.
Storage:	4 °C

Images



ELISA

Image 1.