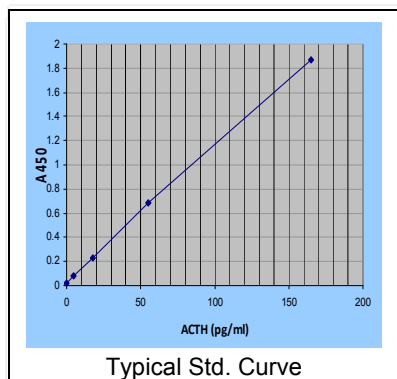


AdrenoCorticotropin Hormone (ACTH) ELISA Kit, 96 tests, Quantitative # 200-120-ACH

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 HRP-labeled detection antibody binds only the mid-region and N-terminal ACTH 1-24. Standards and samples produce concentration dependent yellow color that is measured at 450nm. Concentrations of ACTH present in the controls and patient samples are determined directly from this curve. This kit is intended for the quantitative determination of ACTH (Adrenocorticotropin Hormone) in human plasma. For in vitro research use only.



ELISA Kit Features

- Streptavidin pre-coated, stabilized, ready-to-use 96-well strip plate, stable for ~12 months.
- Anti-ACTH standards (5-165 pg/ml); positive and negative controls.
- Sample size 200 μ l: Serum or plasma; (diluted).
- 4 hrs 30 minute assay. 3 incubation steps, Sensitivity 0.46 U/ml; Good Recovery and Assay Precision.
- Contains all necessary reagents. Shelf life ~12 months.
- Sample values are calculated from the standard curve

Assay Procedure: Allow all reagents to reach room temperature. Arrange and label required number of strips.

- Step 1.** Pipet 200 μ l each of pre-diluted stds, controls and samples into streptavidin coated plate.
- Step 2.** Dispense 25 μ l of Biotinylated antibody and 25- μ l of enzyme labeled antibody into the wells. Cover the plate and incubate at room temp (22-28°C) for 4 hrs on an orbital shaker (180 rpm).
- Step 3.** Aspirate and wash 5X with 1X wash buffer.
- Step 4.** Add 150 μ l TMB substrate to all wells. Cover and incubate by shaking at RT for 30 min on an orbital shaker (180 rpm).
- Step 5.** Pipet 100 μ l of stop solution into each well and mix gently (blue color turns yellow). Measure absorbance at 450 nm. Determine ACTH conc. in each sample using the standards (results are expressed in pg/ml).
- Step 6.** Plot the A450 values against the ACTH conc and calculate unknown values from the std graph.

Performance characteristics

Precision: Intra-assay (%CV): 3.1-4.2 Inter-assay (%CV): 5.8-6.2% **Recovery:** 90-115% (7-29 pg/ml)
Accuracy: 117 samples were run in the ELISA and an IRMA test. The correlation was 0.995
Sample Dilution and Parallelism: samples diluted 1:2-1:16 (recovery 93-109%) **Expected Values:** 7.9-66.1 pg/ml (n=83)
Specificity: ACTH 1-24 (<0.1%) ACTH 18-39 (<0.1%) alpha-MSH/beta endorphin (<0.01%).
Species reactivity: This kit only detects ACTH in human samples. Other species have not been tested.

General information

ACTH (Adrenocorticotropin hormone) or corticotropin 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 secretion from the anterior pituitary is controlled by both a classical negative feedback control mechanism and CNS-stress mediated control system. Various types of stress or pain perceived in higher levels of the brain modulate secretion of the hypothalamic neurosecretory hormone, corticotropin releasing hormone (CRH), a 41-amino acid peptide. CRH stimulates pituitary ACTH secretion. The second peptide that modulates ACTH secretion is vasopressin (AVP). AVP secretion is also stimulated by stress and acts synergistically with CRH to increase ACTH secretion in the pituitary portal circulation. ACTH increases the synthesis and release of all adrenal steroids, 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. Biologically active ACTH results from enzymatic cleavage of a large precursor molecule, pro-opiomelanocortin (POMC). This molecule contains within its structure the amino acid sequences of ACTH, Pro-ACTH, β -melanocyte stimulating hormone, lipotropin, as well as endorphin and the enkephalins.

Because the reaction in immunoassays is determined by antigenic structure, not biological function, the usual ACTH RIA reacts with POMC, Pro-ACTH, ACTH and some fragments of the ACTH. Like other pituitary hormones, ACTH is secreted in a pulsatile manner. These small pulses are superimposed on a characteristic diurnal fluctuation of greater amplitude. In healthy individuals, ACTH reaches a peak in the early morning (6:00 - 8:00 hour) and levels become lowest late in the day and near the beginning of the sleep period. Because of this diurnal rhythm it is customary to draw plasma ACTH samples between 8:00 and 10:00 hour. However, differentiation of patients with Cushing's disease from normal individuals may be best achieved on samples obtained in the evening (16:00 - 18:00 hour). In Cushing's disease and in ectopic ACTH syndromes, the diurnal pattern of ACTH secretion is generally absent. Stress may also override the diurnal variation.

200-120-ACH-flr Rev.140602A

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