# Fast Adrenocorticotropic Hormone /ACTH ELISA

For the quantitative determination of human ACTH concentrations in cell culture supernates, serum, and plasma.

## INTRODUCTION

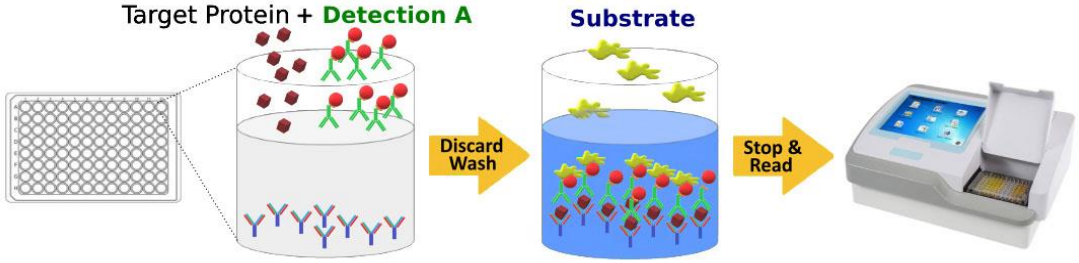
Adrenocorticotropic hormone (ACTH, also adrenocorticotropin, corticotropin) is a 39 amino acid polypeptide hormone (MW=4,500) secreted by the pituitary. ACTH is important for the hypothalamic-pituitary-adrenal axis (HPA) produced in response to biological stress. Its principal effects are for regulation of production and release of glucocorticoids (GCs). Stress-induced secretion of the peptide hormone Corticotropin Releasing Hormone (CRH) stimulates pituitary ACTH secretion. Circulating ACTH binds to melanocortin receptors on the surface of adrenal zona cells, which induce the synthesis and release of all adrenal steroids, aldosterone, GCs and adrenal androgens. ACTH is the principal modulator of cortisol and corticosterone, considered the most important glucocorticoids in higher organisms. Plasma ACTH levels are a useful biomarker for diagnosis of ACTH disorders.

Tribioscience's Fast ACTH ELISA is designed to quantitatively detect ACTH levels in serum, plasma, and other biological samples with our FAST proprietary approaches to combine samples and detections into a one-step. It makes the assay simple, easy, accurate and fast. The total operating time can be finished within 2 hours, not need 4-5 hours (Fig. 1). The detection range is from 7 to 500 pg/mL. The ACTH levels of samples are parallel to the standard curves obtained using the kit standards linearly. Therefore, the kit can be used to determine relative mass values for ATCH.

## PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique (See Fig. 1). A monoclonal antibody specific for ACTH was pre-coated onto a microplate. Standards or samples and Detection Antibody are pipetted into the wells, and concurrently incubated for 1-2 hours. Then, just aspirate each well, no wash, directly add Secondary Antibody, incubate the complex. Following a wash to remove any unbound antibody and samples, an **ultra-sensitive TMB substrate solution** is added to the wells for color develops. The color intensity is in proportion to the amount of bound in the initial step. The intensity of the color is measured by plate read at 450 nm.

**Fig.1**




## KIT CONTENT AND STORAGE CONDITIONS one 96 well plate

| PART | PART# | DESCRIPTION | STORAGE OF OPENED/ RECONSTITUTED |
|---|---|---|---|
| Human ACTH Microplate | TBS3290A | 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for ACTH. | Return unused wells to the foil pouch. Reseal along the entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C. |
| Human ACTH Standard | TBS3290B | 60 µl of Recombinant ACTH (10 ng/mL). | Aliquot and store at -20 °C for up to 1 month in a manual defrost freezer. Avoid repeated freeze-thaw cycles. |
| Detection A | TBS3290C | 2.1 ml of ACTH Detection antibody. | May be stored for up to 4 months at 2-8 °C. |
| Assay Diluent | TBS3290D | 12 ml of a buffered protein base with preservatives. | |
| Wash Buffer | TBS3000W | 12 ml of concentrated solution (10x). | |
| TMB Substrate | TBS3000T | 12 ml of ultra-sensitive TMB substrate. | |
| Stop Solution | TBS3000S | 6 ml of 2 N sulfuric acid. | |

Store the unopened kit at 2-8 ℃. Do not use past kit expiration date. The kit is sufficient for one 96-well plate ELISA.

## PRECAUTIONS

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

## REAGENT PREPARATION

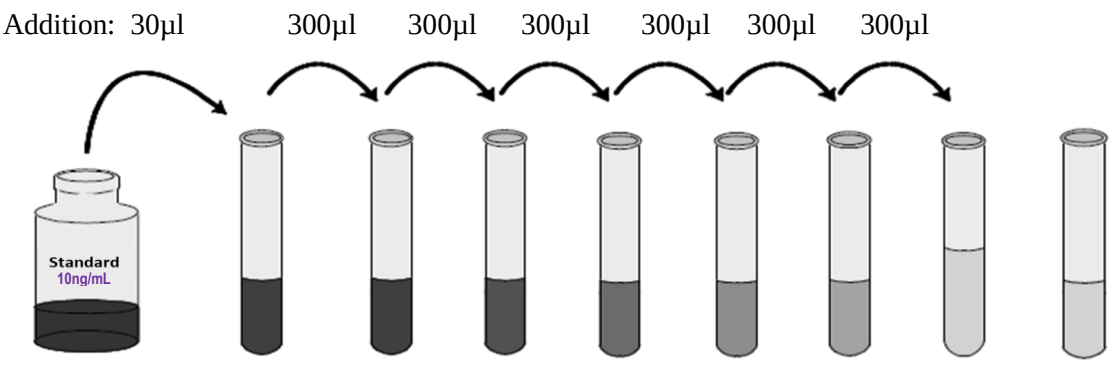
**Bring all reagents to room temperature before use.**

**Wash Buffer:** Add 12 mL of Wash Buffer Concentrate (10x) to 108 mL of deionized distilled water to prepare 120 mL of Wash Buffer (*If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved).*

**ACTH Standard Preparation:** Label test tubes as #1 through #8. Pipet 570 µL of 1x Assay Diluent into tube #1, and 300 µL into tubes #2 to #8 **as diagram below.**

1. Add 30 µL of the ACTH Standard stock solution (10ng/mL) to tube #1 and mix.
2. Make 2x serial dilutions of the standard using the Tube#1(500 pg/mL standard solution) from Tube #2 through #7 with sequential transfer of 300 µL to the next concentration. Mix each tube thoroughly before the next transfer. The standard concentration in tube 1 through 7 will be 500, 250, 125, 62.5, 31.25, 15.625 and 7.8125 pg/mL. Tube# 8 is Standard 0.

**Fig.2 Diagram for ACTH standard preparation**




| | Std1 | Std2 | Std3 | Std4 | Std5 | Std6 | Std7 | Std8 |
|---|---|---|---|---|---|---|---|---|
| **Assay Buffer (µL)** | 570 | 300 | 300 | 300 | 300 | 300 | 300 | 300 |
| **Addition** | Stock | Std1 | Std2 | Std3 | Std4 | Std5 | Std6 | |
| **Addition Vol. (µL)** | 30 | 300 | 300 | 300 | 300 | 300 | 300 | 0 |
| **Final Conc (pg/mL)** | 500 | 250 | 125 | 62.5 | 31.25 | 15.625 | 7.8125 | 0 |

## ASSAY PROCEDURE

**Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.**

1. Add 80 µL of standard, sample, or control per well.
2. Add 20 µL of **Detection A** to the above standard and sample of each well, thoroughly mix. Cover with the adhesive sealer. Incubate at **RT for 2 hours**.
3. Aspirate each well, and wash for 3 times by filling each well with 250 µL Wash Buffer (*Complete removal of liquid at each step is essential to good performance*). After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add 100 µL of **TMB Substrate** to each well. Incubate **at RT for 10-20min** (*Protect from light*). The color becomes blue.
5. Add 50 µL of **Stop Solution** to each well. The color in the well should change from blue to yellow (gently tap the plate to ensure thorough mixing).
6. Determine the optical density of each well within 20 minutes, using a microplate reader at 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the

plate. Readings made directly at 450 nm without correction may be higher and less accurate.

## CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample subtract the average zero standard optical density (O.D.).

Create a standard curve using computer software capable of generating a four-parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the Y-axis against the concentration on the X-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

## TYPICAL DATA

This standard curve($R^2$=0.999) is provided for demonstration only. A standard curve should be generated for each set of samples assayed. Fig. 3 is an example of typical Data.

## SENSITIVITY

The minimum detectable dose (MOD) of ACTH is typically 5 pg/ml.
The Intra-assay CV and the Inter-assay CV are <10%.

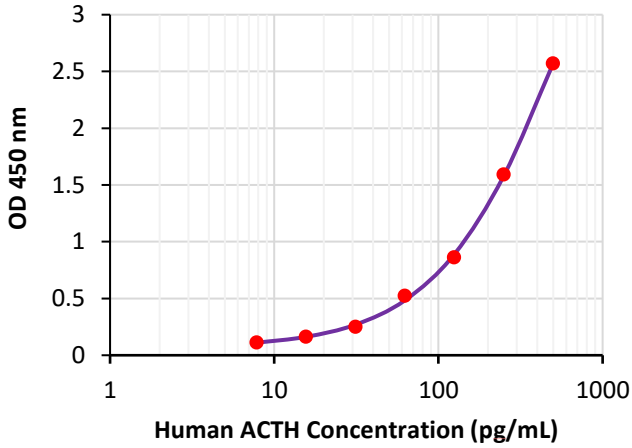
## SPECIFICITY

This assay recognizes natural and recombinant human ACTH.
No cross-reactivity with others.

## RELATIVE PRODUCTS

Human NF-L ELISA (TBS32101)
Human IL-2 ELISA (TBS3220)
Human IL-4 ELISA (TBS3221)
Human IL-6 ELISA (TBS3223)
Human IL-7 ELISA (TBS3224)
Human IL-8 ELISA (TBS3225)
Human IL-10 ELISA (TBS3226)
Human IL-13 ELISA (TBS3227)
Human IL-17 ELISA (TBS3228)
Human IL-22 ELISA (TBS3229)
Human IL-33 ELISA (TBS4245)
Human IFN-gamma ELISA (TBS3230)
Human TGF- ß1 ELISA (TBS3232)
Human GM-CSF ELISA (TBS3233)
Human MIP-1α ELISA (TBS3234)
Protein Cell Lysis Buffer (catalog# TBS5001)
Protein Assay Kit (Catalog# TBS2005)
TMB Substrate System (Catalog#TBS5021)

**Fig.3 Human ACTH Standard Curve**

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