



**EAGLE**  
BIOSCIENCES

# ACTH ELISA Kit

Catalog Number:

**ACT31-K01 (1 x 96 wells)**

For Research Use Only. Not for use in diagnostic procedures.

*v. 6 (effective 30APR17)*

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## INTENDED USE

The Eagle Biosciences ACTH ELISA Kit is intended for the quantitative determination of human adrenocorticotrophic hormone (ACTH) in EDTA-plasma. The ACTH ELISA Kit is for research use only and not to be used in diagnostic procedures.

*For further information about this kit, its application, or the procedures in this insert, please contact the Technical Service Team at Eagle Biosciences, Inc at [www.EagleBio.com](http://www.EagleBio.com) or at 866-411-8023.*

## INTRODUCTION

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.

## PRINCIPLE OF THE ASSAY

The Eagle Biosciences ACTH 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 two selected monoclonal antibodies that bind to N-terminal and C-terminal epitopes of ACTH.

Assay standards, controls and samples are added directly to wells of microplate 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 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 point-to-point, or 4 parameter curve fitting. The concentration of human ACTH in test samples is determined directly from this standard curve.

## LIMITATIONS RELATED TO INTENDED USE

- This ACTH ELISA Kit requires EDTA-plasma sample for testing. Serum samples may show a lower ACTH level and must not be used because ACTH is not stable in serum.
- Because of 24 hour circadian rhythms of circulating ACTH levels, the time of day the sample was collected should be considered when interpreting test results. Therefore, a normal ACTH test result doesn't rule out related conditions.
- For sample values reading greater than highest standard, it is recommended to re-assay samples with dilution (i.e. 1:10 or 1:100 with standard zero).
- Water deionized with polyester resins may inactivate the horseradish peroxidase enzyme.

## PROCEDURAL WARNINGS AND PRECAUTIONS

- This kit is for use by trained laboratory personnel (professional use only). For research use only.
- Practice good laboratory practices when handling kit reagents and specimens. This includes:
- Do not pipette by mouth.
- Do not smoke, drink, or eat in areas where specimens or kit reagents are handled.
- Wear protective clothing and disposable gloves.
- Wash hands thoroughly after performing the test.
- Avoid contact with eyes, use safety glasses; in case of contact with eyes, flush eyes with water immediately and contact a doctor.
- Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
- Do not use this kit beyond the expiry date stated on the label.
- If the kit reagents are visibly damaged, do not use the test kit.
- Do not use kit components from different kit lots within a test and do not use any component beyond the expiration date printed on the label.
- All kit reagents and specimens must be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of specimens.
- When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
- Immediately after use, each individual component of the kit must be returned to the recommended storage temperature stated on the label.
- A calibrator curve must be established for every run.
- It is recommended to all customers to prepare their own control materials or sample pools which should be included in every run at a high and low level for assessing the reliability of results.
- The controls (if applicable with this kit) must be included in every run and their results must fall within the ranges stated in the quality control certificate; a failed control result might indicate improper reagent storage.
- When dispensing the substrate and stopping solutions, do not use pipettes in which these liquids will come into contact with any metal parts.
- The TMB Substrate is sensitive to light and should remain colorless if properly stored. Instability or contamination may be indicated by the development of a blue color, in which case it should not be used.
- Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
- Samples or controls containing azide or thimerosal are not compatible with this kit, they may lead to false results.
- Avoid microbial contamination of reagents.
- To prevent the contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, calibrator, and control.
- To prevent contamination of reagents, do not pour reagents back into the original containers.
- Kit reagents must be regarded as hazardous waste and disposed of according to local and/or national regulations.
- Consumables used with the kit that are potentially biohazardous (e.g., pipette tips, bottles or containers containing human materials) must be handled according to biosafety practices to minimize the risk of infection and disposed of according to local and/or national regulations relating to biohazardous waste.
- This kit contains acid in the stopping solution component. Do not combine acid with waste material containing sodium azide or sodium hypochlorite.

- The use of safety glasses, and disposable plastic, is strongly recommended when manipulating biohazardous or bio-contaminated solutions.
- Proper calibration of the equipment used with the test, such as the pipettes and absorbance microplate reader, is required.
- If a microplate shaker is required for the assay procedure, the type and speed of shaker required is stated in the REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED section. Both the type and speed of shaker used can influence the optical densities and test results. If a different type of shake and/or speed is used, the user is responsible for validating the performance of the kit.
- Do not reuse the microplate wells, they are for SINGLE USE only.
- To avoid condensation within the microplate wells in humid environments, do not open the pouch containing the microplate until it has reached room temperature.
- Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the participant is established.
- When reading the microplate, the presence of bubbles in the wells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.

## **SAFETY CAUTIONS AND WARNINGS**

### **BIOHAZARDS**

The reagents should be considered a potential biohazard and handled with the same precautions applied to human specimens. All human specimens should be considered a potential biohazard and handled as if capable of transmitting infections and in accordance with good laboratory practices.

### **CHEMICAL HAZARDS**

Avoid direct contact with any of the kit reagents. Specifically avoid contact with the TMB Substrate (contains tetramethylbenzidine) and Stopping Solution (contains sulfuric acid). If contacted with any of these reagents, wash with plenty of water and refer to SDS for additional information.

## **SPECIMEN COLLECTION, STORAGE, AND PRE-TREATMENT**

### **Specimen Collection & Storage**

Since the circulation ACTH shows a 24 hours circadian rhythms, it is recommend drawing blood sample early morning or before 8 a.m. Subjects 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. Totally 0.4 mL EDTA-plasma is required for duplicate determination of human ACTH with this test kit. Whole blood should be collected with lavender-top Vacutainer and the plasma separated according to manufacturer's instruction. The EDTA-plasma should be separated from the 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.

## **REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED**

- Precision single channel pipettes capable of delivering 25 µL, 200 µL, etc.
- Disposable pipette tips suitable for above volume dispensing
- Aluminum foil
- Deionized or distilled water.

- Plastic microtiter well cover or polyethylene film.
- ELISA multichannel wash bottle or automatic (semi-automatic) washing system
- Spectrophotometric microplate reader capable of reading absorbance at 450/650 or 450/620 nm.

## REAGENTS PROVIDED

### 1. Anti-ACTH Antibody Coated Microplate

Contents: One 96-well (12 x 8) microplate coated with polyclonal anti-human ACTH antibody.

Format: Ready to Use

Storage: 2-8°C

Stability: Stable until the expiry date printed on the label.

### 2. HRP Conjugated Anti-ACTH Tracer Antibody

Contents: One bottle containing HRP-labeled anti-human ACTH antibody in a stabilized protein matrix.

Format: Concentrated; Requires Preparation

Volume: 0.25 mL/bottle

Storage: 2-8°C

Stability: Unopened: Stable until the expiry date printed on the label.  
After Preparation: Discard after use.

Preparation: **Dilute 1:21** with the Tracer Antibody Diluent. Following is a table that outlines the relationship of strips used and antibody mixture prepared.

Dilution Scheme	Tracer Antibody Diluent	Tracer Antibody
1	0.4 mL	20 µL
2	0.8 mL	40 µL
3	1.2 mL	60 µL
4	1.6 mL	80 µL
5	2.0 mL	100 µL
6	2.4 mL	120 µL
7	2.8 mL	140 µL
8	3.2 mL	160 µL
9	3.6 mL	180 µL
10	4.0 mL	200 µL
11	4.4 mL	220 µL
12	4.8 mL	240 µL

### 3. **ELISA HRP Substrate**

Contents: One bottle containing tetramethylbenzidine (TMB) with hydrogen peroxide.  
Format: Ready to Use  
Volume: 25 mL  
Storage: 2-8°C  
Stability: Stable until the expiry date printed on the label

### 4. **ELISA Wash Concentrate (30x)**

Contents: One bottle of concentrated wash buffer containing a surfactant in phosphate buffered saline with non-azide, non-mercury based preservative.  
Format: Concentrated; Requires Preparation  
Volume: 30 mL/bottle  
Storage: 2-25°C  
Stability: Unopened: Stable until the expiry date printed on the label.  
Preparation of Working Solution: **Dilute 1:30.** Combine the contents with 870 mL of distilled or deionized water and mix well before use.

### 5. **ELISA Stop Solution**

Contents: One bottle containing an acidic stopping solution.  
Format: Ready to Use  
Volume: 12 mL/bottle  
Storage: 2-8°C  
Stability: Stable until the expiry date printed on the label.

### 6. **Human ACTH Standards**

Contents: Six bottles containing human ACTH in a lyophilized bovine serum-based matrix with a non-azide preservative. Refer to the vials for exact concentrations of the standard.  
Format: Lyophilized; Requires Reconstitution  
Storage: 2-8°C, -10°C (Long-Term Storage)  
Stability: Unopened: Stable until the expiry date printed on the label.  
After Preparation: 24 hours at room temperature, aliquoted and frozen for long-term storage. Do not exceed 3 freeze-thaw cycles  
Preparation: Add 2.0 mL of distilled or deionized water, allow to sit for 5 minutes undisturbed, then mix well by inversions or gently vortexing. Make sure all solids are dissolved completely prior to use.

### 7. **Human ACTH Controls**

Contents: Two bottles containing human ACTH in a lyophilized bovine serum-based matrix with a non-azide, non-mercury based preservative. Refer to bottles for exact concentration.  
Format: Lyophilized; Requires Reconstitution  
Storage: 2-8°C, -10°C (Long-Term Storage)

Stability Unopened: Stable until the expiry date printed on the label.  
After Preparation: 24 hours at room temperature, aliquoted and frozen for long-term storage. Do not exceed 3 freeze-thaw cycles

Preparation: Add 2.0 mL of distilled or deionized water, allow to sit for 5 minutes undisturbed, then mix well by inversions or gently vortexing. Make sure all solids are dissolved completely prior to use.

### 8. Tracer Antibody Diluent

Contents: One bottle containing ready-to-use buffer. It should be used only for tracer antibody dilution.

Format: Ready to Use

Storage: 2-8°C

Stability: Stable until the expiry date printed on the label.

### RECOMMENDED ASSAY LAYOUT\*

	1	2	3	4	5	6	7	8	9	10	11	12
A	STD 1	STD 1	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample
B	STD 2	STD 2	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample
C	STD 3	STD 3	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample
D	STD 4	STD 4	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample
E	STD 5	STD 5	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample
F	STD 6	STD 6	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample
G	control 1	control 1	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample
H	control 2	control 2	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample

\*Layout subject to change based on standard and control quantities

### ASSAY PROCEDURE

All kit components, controls, and specimen samples must reach room temperature prior to use. Standards, controls, and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. Add **200 µL of standards, controls and samples** into designated wells.
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 hours ± 5 minutes at 400 to 450 rpms**.
4. Remove plate sealer. Wash each well 5 times by dispensing **350 µL** of working wash solution into each well, and then completely aspirate the contents. Alternatively, an automated microplate washer can be used.
5. Add **200 µL** of 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 **100 µL** of Stop Solution into each of the wells. Mix gently.
8. Read the absorbance at **450 nm** with reference filter at 620 nm or 650.

### CALCULATIONS

It is recommended to use a point-to-point or 4-parameter standard curve fitting.

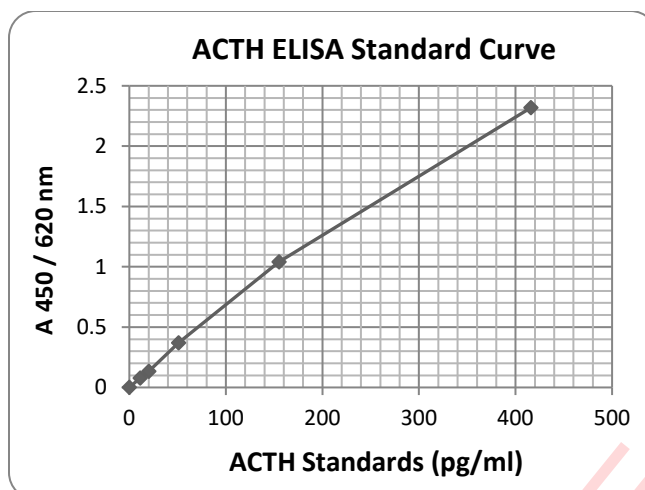
1. Calculate the average absorbance for each pair of duplicate test results.
2. Subtract the average absorbance of the level 1 standard (0 ng/mL) from the average absorbance of all other readings to obtain corrected absorbance.
3. The standard curve is generated by the corrected absorbance of all standard 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 subject samples are read directly from the standard curve using their respective corrected absorbance.

### EXAMPLE DATA AND STANDARD CURVE

A typical absorbance data and the resulting standard curve from this ACTH ELISA Kit are represented. This curve should not be used in lieu of standard curve run with each assay.

Well I.D.	OD 450/650 nm Absorbance			Results
	Readings	Average	Corrected	
Std-1: 0 pg/mL	0.025 0.029	0.027	0.000	
Std-2: 11 pg/mL	0.107 0.102	0.105	0.074	
Std-3: 20 pg/mL	0.160 0.160	0.160	0.133	
Std-4: 51 pg/mL	0.400 0.395	0.398	0.371	
Std-5: 155 pg/mL	1.057 1.081	1.069	1.042	
Std-6: 416 pg/mL	2.316 2.375	2.346	2.319	
Control 1	0.244 0.260	0.252	0.225	32.0 pg/mL
Control 2	0.676 0.714	0.695	0.668	97.1 pg/mL



### EXPECTED VALUES

EDTA plasma samples from normal healthy adults ages 20 – 60 were collected and measured with this ELISA. The recommended **normal range** for ACTH concentration by using this ELISA is between 1 – 72 pg/mL. We strongly recommend for each laboratory to establish its own normal range by measuring EDTA plasma samples with this ELISA. Please note that sample collection time of the day may have impact on the ACTH normal range.

### QUALITY CONTROL

To assure the validity of the results each assay should include adequate controls.

### PERFORMANCE CHARACTERISTICS

#### Sensitivity

The analytical sensitivity (LLOD) of the ACTH ELISA Assay kit as determined by the 95% confidence limit on 8 replicate determination of zero standard is less than 1 pg/mL.

#### High Dose “hook” effect

This assay has showed that it did not have any high dose “hook” for ACTH level up to 10,000 pg/mL.

#### Precision

The intra-assay precision was validated by measuring three control samples with 16 replicate determinations.

Sample #	Mean ACTH Value (pg/mL)	CV (%)
1	36.1	7.6
2	66.5	8.6
3	276.9	10.3

The inter-assay precision was validated by measuring two control levels in duplicate in 16 individual assays.

Sample #	Mean ACTH Value (pg/mL)	CV (%)
1	32.1	7.1
2	261.0	5.3



### Linearity

Two ACTH standard levels were diluted with assay buffer and tested. The results of ACTH percent recovery value in pg/mL are as follows:

DILUTION	OBSERVED Value (pg/mL)	RECOVERY %
<b>Neat A</b>	416	-
1:2	231.4	111
1:4	102.1	98
1:8	52.1	100
1:16	26.3	101
<b>Neat B</b>	155	-
1:2	80.3	104
1:4	38.6	100
1:8	20.5	106
1:16	9.3	96

Two EDTA plasma samples were collected and spiked with a high ACTH standard and tested. The results of ACTH percent recovery value in pg/mL are as follows:

DILUTION	OBSERVED VALUE (pg/mL)	RECOVERY %
<b>Neat A</b>	184.1	-
1:2	105.8	115
1:4	58.5	127
1:8	22.6	98
<b>Neat B</b>	33.3	-
1:2	17.1	103
1:4	9.4	113
1:8	5.1	121

## Spike and Recovery

Two EDTA plasma samples and three assay standards (45, 135 and 405 pg/mL) were combined at equal volumes and tested. The results are as follows:

DILUTION	OBSERVED VALUE (pg/mL)	EXPECTED VALUE (pg/mL)	RECOVERY %
<b>Neat A</b>	12.5	-	-
Std-4	25.1	28.8	87
Std-5	73.5	73.8	100
Std-6	254.0	208.8	122
<b>Neat B</b>	21.5	-	-
Std-4	26.1	33.3	79
Std-5	66.8	78.3	85
Std-6	208.1	213.3	98

## REFERENCES

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## WARRANTY INFORMATION

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*For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at [info@eaglebio.com](mailto:info@eaglebio.com) or at 866-411-8023.*

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