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# Product Information

**Mouse/Rat Adrenocorticotropic Hormone (ACTH) ELISA**

Catalog Number **SE120080**
Storage Temperature 2–8 °C

## TECHNICAL BULLETIN

### Product Description

Adrenocorticotropic Hormone (ACTH) is a 39 amino acid peptide hormone (4,500 Da) secreted mainly by the anterior pituitary gland. Various types of stress or pain perceived in higher levels of the brain modulate secretion of the hypothalamic neurosecretory hormone, corticotropin releasing hormone (CRH). 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.

The Mouse/Rat Adrenocorticotropic Hormone (ACTH) ELISA is intended for the quantitative determination of ACTH (Adrenocorticotropic Hormone) in mouse/rat plasma. It is a two-site ELISA (Enzyme-Linked ImmunoSorbent Assay) for the measurement of the biologically active 39 amino acid chain of ACTH. A goat polyclonal antibody to ACTH, purified by affinity chromatography and a mouse monoclonal antibody to ACTH are specific for well-defined regions on the ACTH molecule. 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 horseradish peroxidase [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 substrate, tetramethylbenzidine (TMB). An acidic stopping 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 versus concentration is generated using results obtained from the calibrators. Concentrations of ACTH present in the controls and samples are determined directly from this curve.

### Components

| Materials Provided | 96 Tests |
|---|---|
| Microwells coated with Streptavidin | 6 x 2 x 8 |
| ACTH Standard Zero: 1 bottle, Ready to use | 4 mL |
| ACTH Standards: 6 bottles (Lyophilized) | 2 mL |
| Biotinylated ACTH Antibody (Reagent 1) | 2.7 mL |
| Enzyme labeled ACTH Antibody (Reagent 2) | 2.7 mL |
| TMB Substrate (Reagent B) | 15 mL |
| Stop Solution | 20 mL |
| Wash Concentrate (Reagent A) | 30 mL |

### Reagents and Equipment Required but Not Provided.

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450 nm
5. Absorbent paper or paper towel
6. Graph paper

### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

### Preparation Instructions

Sample Preparation

1. EDTA plasma should be used.
2. No special pretreatment of sample is necessary.
3. Plasma samples may be stored at 2–8 °C for up to 8 hours and should be frozen at –20 °C or lower for up to 4 months. Do not use grossly hemolyzed or grossly lipemic specimens.
4. Samples containing sodium azide should not be used in the assay.

Preparation of non-zero standards/calibrators
For each of the non-zero standards/calibrators (Calibrator B through F), reconstitute each vial with 2 mL of distilled or deionized water, and mix. Allow the vial to stand for 10 minutes and then mix thoroughly by gentle inversion to ensure 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. Standards and controls are stable at –20 °C for 6 weeks after reconstitution with up to 3 freeze thaw cycles.

20x Wash Buffer Concentrate
Prepare 1x wash buffer by adding the contents of the bottle to 475 mL of distilled water. Store 1x wash buffer at room temperature

**Storage/Stability**
Store Kit at 2–8 °C.

**Procedure**
Notes: The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

It is recommended that standards, control, and serum samples be run in duplicate.

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.

Prior to assay, bring all reagents to room temperature. Gently mix all reagents before use.

1. Secure the desired number of coated wells in the holder.
2. Add 200 µL of standards or calibrators, specimens, and controls into appropriate wells. Freeze (–20 °C) the remaining calibrators and controls as soon as possible after use.
3. Add 25 µL of Reagent 1 (Biotinylated Antibody) to each well.
4. Add 25 µL of Reagent 2 (Enzyme labeled antibody) to each well.
5. Cover the plate with aluminum foil to avoid exposure to light and Incubate for 4 hours at room temperature (18–26 °C) with shaking.
6. Remove liquid from all wells. Wash wells five times with 300 µL of 1x wash buffer. Blot on absorbent paper towels.
7. Add or dispense 150 µL of the ELISA Reagent B (TMB Substrate) into each of the wells
8. With appropriate cover to avoid light exposure. Incubate for 30 minutes at room temperature with shaking.
9. Add or dispense 100 µL of the Stopping Solution into each of the wells. Mix gently.
10. Read the absorbance of the solution in the wells within 10 minutes, 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 ~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.
11. By using the final absorbance values obtained in the previous step, construct a calibration curve via cubic spline, 4 parameter logistics, or point-to-point interpolation to quantify the concentration of the ACTH.

**Results**

Calculations

1. For the 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 zero calibrator and the three highest concentrations (i.e., Calibrators A, D, E, and F).

2. 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 Y-axis.

3. 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.

Note: This 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.

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 samples may not be valid.

**Product Profile**

Sensitivity:

The sensitivity of this assay is defined as the smallest single value, which can be distinguished from zero at the 95% confidence limit. The ACTH ELISA has a calculated sensitivity of 0.05 pg/mL.

Correlation:

Eighty samples, with ACTH values ranging from 1.5–1045 pg/mL, were assayed by the ACTH ELISA and a reference ELISA method.

| Correlation | Slope | Intercept |
|---|---|---|
| 0.94 | 0.98 | 0.8 |

Precision and Reproducibility:

Intra-Assay Variation

The precision (intra-assay variation) of the ACTH ELISA Test was calculated from 20 replicate determinations on each of the two samples.

| Sample | Mean Value (pg/mL) | N | Coefficient of Variation % |
|---|---|---|---|
| A | 27 | 20 | 6.7 |
| B | 320 | 20 | 5.6 |

Inter-Assay Variation

The total precision (inter-assay variation) of the ACTH ELISA test was calculated from data on two samples obtained in 20 different assays, by three technicians on three different lots of reagents, over a nine week period

| Sample | Mean Value (pg/mL) | N | Coefficient of Variation % |
|---|---|---|---|
| A | 27 | 20 | 9.8 |
| B | 320 | 20 | 7.6 |

Linearity:

Two samples were diluted with Calibrator A (Zero Calibrator). Results in pg/ml are shown below**:**

| Sample | Dilution | Expected | Observed | % Observed Expected |
|---|---|---|---|---|
| A | Undiluted | 500 | 480 | 96 |
| | 1:2 | 250 | 243 | 97 |
| | 1:4 | 125 | 121 | 96 |
| | 1:8 | 62 | 59 | 95 |
| B | Undiluted | 100 | 105 | 105 |
| | 1:2 | 50 | 46 | 92 |
| | 1:4 | 25 | 22 | 88 |
| | 1:8 | 12 | 10 | 83 |

**References**


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