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Product Information

Mouse/Rat Adrenocorticotropic Hormone (ACTH)
Ultra Sensitive CELISA

Catalog Number **SE120081**
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 ACTH Chemiluninescence ELISA (CELISA) is an ultra sensitive method (Less than 1 pg/mL) intended for the quantitative determination of ACTH in mouse/ rat plasma. The ACTH Immunoassay is a two-site CELISA (Chemiluninescence 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. Upon the addition of the luminol substrate, the enzyme activity in the enzyme-bound fraction is directly proportional to the concentration of the ACTH in the sample. A standard curve is prepared relating light units (RLU) to the concentration of the ACTH. 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: 5 bottles (Lyophilized)	2 mL
Biotinylated ACTH Antibody (Reagent 1)	2.7 mL
Enzyme labeled ACTH Antibody (Reagent 2)	2.7 mL
Luminol substrate, 3x: 1 bottle	4 mL
Luminol buffer: 1 bottle	8 mL
Sample Diluent: 1 bottle	10 mL
Wash Concentrate (Reagent A)	25 mL

Reagents and Equipment Required but Not Provided.

- Distilled or deionized water
- Precision pipettes
- Disposable pipette tips
- Microplate luminometer
- Absorbent paper or paper towel
- 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 (standards 2–6), 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.

3x Luminol Substrate
Prepare 1x Substrate solution by adding 1 part of Luminol to 2 parts Luminol buffer as needed.

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

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 sample may not be valid.

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 2 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 100 µL of luminol substrate to all wells. Read the relative light units in each well using a luminometer (0.2–1 second integration time) within 5 minutes of substrate addition.

Results

Calculations

The standard curve is constructed as follows:

1. Check ACTH standard value on each standard vial. This value might vary from lot to lot. Make sure the value is checked on every kit.
2. To construct the standard curve, plot the RLU (Relative Light Units) for each ACTH standard point (vertical axis) versus the ACTH standard concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points.
3. Read the concentration for controls and each unknown sample from the curve. Record the value for each control or unknown sample.

   Note: The ACTH CELISA 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.

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 CELISA has a calculated sensitivity of less than 1 pg/mL.

Correlation

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

Correlation	Slope	Intercept
0.94	0.98	0.8

Precision and Reproducibility

The precision (intra-assay variation) of the ACTH CELISA 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

The total precision (inter-assay variation) of the ACTH CELISA 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 Standard 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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