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Product Information

Mouse/Rat Adrenocorticotropic Hormone (ACTH) Ultra-Sensitive lumELISA kit

Catalog Number: EA100851
Storage Temperature: 2 – 8°C

Instruction for Use

Intended Use

The ACTH Chemiluninescence ELISA (lumELISA) is an ultra-sensitive method (Less than 1 pg/mL) intended for the quantitative determination of ACTH (Adrenocorticotropic Hormone) in Mouse/Rat plasma.

Background

Adrenocorticotropic Hormone (ACTH) is a 39-amino acid peptide hormone (MW=4500) 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.

Principle of the test

The ACTH Immunoassay is a two-site lumELISA (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 unit (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
1. Microwells coated with Streptavidin	6x2x8
2. ACTH Standard Zero: 1 bottle (Ready to use)	4 mL
3. ACTH Standards:5 bottles (Lyophilized)	2 mL
4. Biotinylated ACTH Antibody (Reagent 1)	2.7 mL

5. Enzyme labeled ACTH Antibody (Reagent 2)	2.7 mL
6. Luminol substrate, 3X: 1 bottle	4 mL
7. Luminol buffer: 1 bottle	8 mL
8. Sample Diluent: 1 bottle	10 mL
9. Wash Concentrate (Reagent A)	25 mL

Materials 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 450nm
5. Absorbance paper or paper towel
6. Graph paper

Disclaimer

This product is for research use only and not intended for diagnostic procedures.

Specimen Collection Handling

1. Collect blood specimens and separate the serum immediately.
2. Plasma should be treated with EDTA.
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.
4. Avoid multiple freeze-thaw cycles.
5. Prior to assay, frozen sera should be completely thawed and mixed well.
6. Do not use grossly hemolyzed or lipemic specimens.

Reagent Preparation

1. For each of the non-zero standards (Standard 2 through 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 insure 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.
2. 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.
3. 3X Luminol Substrate: Prepare 1X Substrate solution by adding 1 part of Luminol to 2 parts Luminol buffer as needed.

Assay Procedure

- Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (18-26°C). Gently mix all reagents before use
- 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
- Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities

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.
8. Read the relative light units in each well using Luminometer (0.2-1 second integration time) within 5 minutes of substrate addition.

Calculation of Results

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 you check the value on every kit. See example of the standard curve.
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.

Example of Standard Curve

	Conc. (pg/ml)	RLU
Std 1	0	5062
Std 2	7	46998
Std 3	18	105622
Std 4	70	391978
Std 5	215	1115350
Std 6	515	2578258

Quality Control

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 patient sample may not be valid.

Limitation of the Procedure

The ACTH lumELISA 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 re-assayed for correct values. Like any analyte used as a diagnostic adjunct, ACTH results must be interpreted carefully with the overall clinical presentations and other supportive diagnostic tests.

References

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