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2017-03-31

Calbiotech Inc.,

CALBIOTECH
A life science company

Mouse/Rat ADRENOCORTICOTROPIC HORMONE (ACTH) *"Ultra Sensitive"* lumELISA

Catalog No. AC562T-100 (96 tests)

INTENDED USE

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

MATERIAL PROVIDED	96 TESTS
Microwells coated with Streptavidin	12x8x1
ACTH Standard Zero: 1 bottle, Ready to use	4mL
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	10mL
Wash Concentrate	25mL

MATERIAL NOT PROVIDED:

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. Microplate luminometer
5. Absorbance paper or paper towel
6. Graph paper

WARNINGS AND PRECAUTIONS

1. For Research Use Only. Not for use in diagnostic procedures.
2. For laboratory use.
3. Potential biohazardous materials:
   The standard and controls may contain animal/human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984
4. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
5. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
6. It is recommended that standards, control and serum samples be run in duplicate
7. 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.

SPECIMEN COLLECTION/HANDLING

1. EDTA plasma should be used.
2. No special pretreatment of sample is necessary.
3. Typically, 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.

REAGENT PREPARATION AND STORAGE

1. Store Kit at 2-8 °C.
2. 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.
3. 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.
4. 3X Luminol Substrate: Prepare 1X Substrate solution by adding 1 part of Luminol to 2 parts Luminol buffer as needed.

ASSAY PROCEDURE

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 (20-25°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 Luminometer (0.2-1 second integration time) with in 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

LIMITATIONS OF THE PROCEDURE

The CBI 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 reassayed 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.