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Cat#: AC018T-100 (96 tests)
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Mouse/Rat Adrenocorticotropic Hormone (ACTH) ELISA

Catalog No. AC018T-100 (96 tests)

INTENDED USE

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

MATERIAL PROVIDED	96 TESTS
Microwells coated with Streptavidin	6x2x8
ACTH Standard Zero: 1 bottle, Ready to use	4 mL
ACTH Standards: 5 bottles (Lyophilized)	2 mL
Controls 1 & 2 (CTRL) (2 Vials)	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)	30mL

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 calibrator 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/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 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.

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 4 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 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 approximately 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, patient and control 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.

CALCULATION OF RESULTS

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. CalibratorsA, 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 Yaxis.
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.

LIMITATIONS OF THE PROCEDURE

The CBI ACTH 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. Like any analyte used as a diagnostic adjunct, ACTH results must be interpreted carefully with the overall clinical presentations and other supportive diagnostic tests.