ImmuChem™ Double Antibody
hACTH

125I RIA Kit
For In Vitro Diagnostic Use

MP Biomedicals, LLC
Diagnostics Division
13 Mountain View Avenue
Orangeburg, NY 10962

07914061.2
Q03-177, Q05-004 (1/05)

ORDERING INFORMATION

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Price quotations for standing orders and quantity purchases will be supplied upon request. Since we are unable to restock this product, all sales are final. All shipments are F.O.B. Orangeburg, NY

ImmuChem™ hACTH DA
CATALOG No. 07-106101 (50 Tubes)

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CATALOG No. 07-106101 (50 Tubes)

European Authorized Representative:
MP Biomedicals Europe, n.v.-s.a.
Doornveld 10
B-1731 Asse-Relegem, Belgium
Tel: +32 2 466 0000 / Fax: +32 2 466 2642

LICENSING REQUIREMENTS

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INTRODUCTION

A. CLINICAL PHYSIOLOGY

The interrelationship of the pituitary gland and its target organs is demonstrated in a classical fashion by the pituitary-adrenal axis. Pituitary ACTH stimulates the release of adrenal steroids, including cortisol. In turn, cortisol acts in a negative feedback manner to suppress ACTH release. This classical scheme has been amplified in recent years by the demonstration of the hypothalamic Corticotrophin Releasing Factor (CRF). CRF stimulates the release of ACTH from the pituitary gland. Evaluation of disorders of the adrenal gland has depended upon measurement of either urinary cortisol metabolites, or plasma/serum cortisol. The development of a radioimmunoassay for ACTH has expanded diagnostic capabilities. Due to the interrelationship of circulating ACTH and cortisol, simultaneous measurements of these two hormones provide the greatest information.

Adrenal cortical hyperfunction generally occurs from one of three different mechanisms. The first is dysfunction of the hypothalamus or pituitary, leading to excessive ACTH secretion, thus stimulating adrenal hyperplasia. A second mechanism is the ectopic ACTH syndrome from neoplasms or non-pituitary tissue such as oat-cell carcinomas of the lung which secrete ACTH or an ACTH-like peptide. The third cause is excessive cortisol secretion by adrenal tumors, benign or malignant. In this later circumstance, pituitary ACTH secretion is suppressed. The finding of a suppressed value for plasma ACTH levels in the face of elevated cortisol levels suggests primary adrenal neoplasia and augments information obtained from dexamethasone suppression studies. It should be noted that plasma ACTH levels in pituitary mediated Cushing’s syndrome may be normal or only slightly elevated but are disproportionately high in relationship to elevated cortisol levels.

Adrenal hypoplasia can result from primary adrenal failure or can be secondary to pituitary insufficiency. In the case of primary adrenal failure, the negative feedback results in increased ACTH secretion, whereas pituitary insufficiency leads to decreased ACTH levels.

In considering the above principles, a word of caution is in order. Cortisol secretion has been found in recent years to be episodic, although the diurnal variation remains the predominant characteristic. ACTH also appears to be secreted in intermittent bursts. The shorter half-life of ACTH (4-18 minutes) also adds to variability in its level during the day. A variety of stressful situations, both physical and psychological also may cause ACTH release.

Therefore, it may be necessary to measure these hormones on more than one occasion for maximum diagnostic reliability. In addition, provocative tests such as dexamethasone suppression [9,10,11] or Metopirone® stimulation [2,12] aid in evaluating the status of the pituitary-adrenal function.

B. CLINICAL APPLICATIONS

The measurement of peptide hormones as tumor markers appears to have a promising future. ACTH in particular has been found to be produced by many non-pituitary malignancies. At times this occurs in the precursor form, the so-called “Big ACTH”, [13,14] which requires its own specific assay. However, many tumors produce a hormone that is immunologically indistinguishable from pituitary ACTH and is measurable in our assay.

PRINCIPLE OF THE TEST

Radioimmunoassay (RIA) is the term applied to the measurement of the concentration of antigen molecules using a radioactive label that quantitates the amount of antigen (i.e., hormone) by determination of the extent to which it combines with its antibody.

In the assay, a limited amount of specific antibody (Ab) is reacted with the corresponding hormone (*H) labeled with a radioisotope. Upon addition of an increasing amount of the hormone (H), a correspondingly decreasing fraction of *H adds is bound to the antibody. After separation of the bound from the free *H by various means, the amount of radioactivity in one or both of these two fractions is evaluated and used to construct a standard curve against which the unknown samples are measured.

REAGENTS PROVIDED AND LABEL COLOR CODE (50 Tube Kit)

<table>
<thead>
<tr>
<th>COMPONENT</th>
<th>LABEL BAR COLOR</th>
<th>VOLUME OR QUANTITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-ACTH 125I</td>
<td>Yellow</td>
<td>5.5 mL</td>
</tr>
<tr>
<td>Cat. No. 07-106112</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACTH Standards (8)</td>
<td>Green</td>
<td>1 mL ea.</td>
</tr>
<tr>
<td>Cat. No. 07-106130</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACTH Controls (2)</td>
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<td>1 mL ea.</td>
</tr>
<tr>
<td>Cat. No. 07-106180</td>
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<td></td>
</tr>
<tr>
<td>125I hACTH</td>
<td>Blue</td>
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<tr>
<td>Cat. No. 07-106120</td>
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<td></td>
</tr>
<tr>
<td>Precipitant Solution</td>
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<td>27 mL</td>
</tr>
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<td>Cat. No. 07-166623</td>
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</tr>
<tr>
<td>ACTH Water</td>
<td>Tan</td>
<td>30 mL</td>
</tr>
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<td>Cat. No. 07-106163</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*0 pg/mL standard contains 2 mL.

REAGENT DESCRIPTION AND PREPARATION

Some of the reagents in this kit are in the lyophilized form. They should be reconstituted as per the instructions, with the water supplied in the kit. Immediately after use, these reagents should be frozen at -20°C ± 2°C. They may be thawed once, used again and then discarded.

For In Vitro Diagnostic Use
A. ANTI-ACTH

A purified porcine ACTH-conjugate was used to generate this antisera in rabbits. The antisera binds 20-40% of hACTH \( ^{125} \text{I} \) (freshly iodinated) in the absence of nonradioactive hACTH.

Prior to use, reconstitute with 5.5 mL of water (provided) and allow to stand 15 minutes at 4°C ± 2°C. After use, store at -20°C ± 2°C. Thaw only once.

**STORAGE:** Lyophilized at 2 to 8°C. Reconstituted at -20°C ± 2°C.

**STABILITY:** Refer to expiration date on the kit vial.

B. ACTH STANDARDS

Eight human synthetic ACTH-\(^{39}\) standards are provided at the following target concentrations: 0, 10, 25, 50, 100, 250, 500 and 1000 pg/mL in human plasma.* Please refer to the vial label for the exact standard concentration.

Prior to use reconstitute with 1.0 mL (2.0 mL for the 0) of water (provided) and allow to stand 15 minutes at 4°C ± 2°C. After use, store at -20°C ± 2°C. Thaw only once.

**STORAGE:** Lyophilized at 2 to 8°C. Reconstituted at -20°C ± 2°C.

**STABILITY:** Refer to expiration date on vial.

C. ACTH CONTROLS

A high and low concentration of ACTH has been added to human plasma for controls*. Prior to use, reconstitute with 1.0 mL of water (provided) and allow to stand 15 minutes at 4°C ± 2°C. After use, store at -20°C ± 2°C. Thaw only once. Please refer to the vial label for the control ranges.

**STORAGE:** Lyophilized at 2 to 8°C. Reconstituted at -20°C ± 2°C.

**STABILITY:** Refer to expiration date on vial.

*NOTE:* The plasma used to manufacture the standards and controls is of human origin. Although it has been tested and found negative for HIV and HBsAg by an FDA approved method, these standards and controls should be handled with the same safety precautions afforded any human serum/plasma sample.

D. hACTH\(^{125} \text{I}\) TRAER

(Synthetic hACTH\(^{39}\) -) This radioactive material contains less than 0.4 µCi per vial on the date of shipment. 0.1mL of this radioactive material will provide 12,000 to 15,000 cpmp at 75% counter efficiency on the iodination date. This material comes lyophilized and should be reconstituted with 5.5 mL of water (provided) and allow to stand 15 minutes at 4°C ± 2°C before use. After use, store at -20°C ± 2°C. Thaw only once.

**STORAGE:** Lyophilized at 2 to 8°C. Reconstituted at -20°C ± 2°C.

**STABILITY:** Refer to expiration date on kit vial.

E. PRECIPITANT SOLUTION

A mixture of goat anti-rabbit gamma globulins and PEG is contained in a 0.1M Tris buffer. 0.5mL of this mixture will immediately precipitate all the antibody target.

**STORAGE:** Reconstituted at -20°C ± 2°C.

**STABILITY:** Refer to expiration date on kit vial.

NOTE: The plasma used to manufacture these standards and controls is of human origin. Although it has been tested and found negative for HIV and HBsAg by an FDA approved method, these standards and controls should be handled with the same safety precautions afforded any human serum/plasma sample.

F. ACTH WATER

Purified water for reconstituting lyophilized reagents.

**STORAGE:** 2 to 8°C.

**STABILITY:** Refer to expiration date on vial.

V. LIMITATIONS, PRECAUTIONS AND GENERAL COMMENTS

1. These reagents contain sodium azide which has a tendency to build up in lead or copper plumbing forming potentially explosive metal azides. Always flush large quantities of water through the plumbing after the disposal of these reagents. It is recommended to dispose of radioactive waste according to the established U.S. NRC guidelines.

2. This ACTH assay can only be run using EDTA plasma samples. Special collection techniques must be employed when obtaining patients' samples (see section VI Specimen Collection and Handling).

**NOTE:**

A. Strict adherence to the protocol is advised for reliable performance. Any changes or modifications are the responsibility of the user.

B. Do not use glass test tubes for the assay.

C. Care must be taken that clinical samples do not contain any exogenous radioactivity since it may interfere with the results.

D. A standard curve must be established with every assay.

E. The reagents provided in this kit have been designed and optimized for the measurement of ACTH from human plasma. Anyone performing animal research work must establish their own physiological ranges.

F. The use of grossly hemolized or lipemic samples should be avoided.

G. The reagents supplied in this kit are for IN-VITRO DIAGNOSTIC USE.

H. All samples should be collected between 8:00 & 9:00 AM. PM samples should be collected between 4:00 & 5:00 PM.

I. RADIOACTIVE MATERIALS

Please observe the following precautions when handling this radioactive material:

1. This radioactive material may be received, acquired, possessed and used only by those licensed to do so. Its use is solely for in-vitro clinical or laboratory tests not involving the internal or external administration of the materials, i.e. radioactivity, to humans or animals. Its receipt, acquisition, possession, use and transfer are subject to the regulations of, and with a general license from the U.S. NRC or the state with which the U.S. NRC has entered into agreement for the exercise of regulatory authority.

2. Immediately upon the receipt of this kit, check for breakage and verify the contents as per the packing list. Should there be any breakage or questions regarding this kit's contents, immediately notify your distributor by telephone.

3. Kit reagents should be stored and used only at clean, designated work stations of the laboratory. Although the exposure to radiation from the radioactive decay of the small amount of isotope supplied is negligible, it is a good practice to designate a storage area at least 10 feet from any work station. Furthermore, persons under the age of 18 should not be permitted to handle radioactive material or enter into an area where it is either stored or used.

4. Should there be spillage of any of the radioactive material, the following clean-up procedure is recommended: while wearing rubber gloves, blot the spillage with an absorbent material. Dispose of this material as radioactive waste. Remove the gloves, tear to prevent further usage and discard as regular waste. Finally, wash your hands thoroughly.

5. The pipetting of radioactive material by mouth should be avoided. Smoking, eating or drinking while performing tests involving radioactive material should not be permitted. Lastly, people handling radioactive material should wash their hands immediately after handling and prior to leaving the laboratory area.

VI. SPECIMEN COLLECTION AND HANDLING

EDTA PLASMA ONLY!

Prior to drawing blood, chill lavender capped (EDTA) Vacutainers™ to 0° to 4°C. Draw blood into Vacutainers™ and invert gently to mix contents. If drawn blood cannot be centrifuged immediately, place tubes in an ice bath. Because of the short half-life of ACTH in whole blood, samples should be centrifuged within sixty (60) minutes from drawing time. Refrigerated centrifugation is recommended, although a short centrifugation at 20°C should not appreciably affect the sample. Samples stored at 0°C should be used within four (4) hours. Plasma stored at -20°C can be stored for one (1) week. For longer storage, add Trasylol™ (1000 KIU/mL) and store at -72°C. Samples may only be thawed once. The use of Heparin and Sodium Citrate as anticoagulants must be avoided.

VII. EQUIPMENT AND REAGENTS REQUIRED

In addition to the materials provided with this kit, the following supplies are required:

- 1 pipettors and/or pipettes that can accurately and precisely deliver the required volumes (100 µL and 500 µL).
- 2 test tube racks.
- 3 ice bath large enough to accommodate rack.
- 4 laboratory vortex mixer.
- 5 Refrigerated Water Bath or a Refrigerator capable of maintaining 4°C ± 2°C.
- 6 Centrifuge - (capable of 950-1050 x g) (a refrigerated centrifuge is recommended but not required).
- 7 An aspiration or decanting device.
- 8 A gamma counter calibrated for \(^{125} \text{I}\).

*Available from MP BIOMEDICALS

VIII. ASSAY PROCEDURE

A. ASSAY PREPARATIONS

1. Prior to assay, reconstitute lyophilized reagents, mix gently and let sit for 15 minutes at 4°C ± 2°C or allow frozen reagents to thaw in a 4°C ± 2°C environment. Take out vial of precipitant solution and let it sit at room temperature until needed.

2. Set up assay in consecutively numbered polystyrene tubes (provided). Place test tube rack on crushed ice or in an ice bath. All reagents and samples should similarly be placed on the ice or in the water bath. Add the solutions in the order indicated in the protocol (section IX). Pipet all reagents directly from shipping vials.
B. ASSAY STEPS

1. Add diluent water, hACTH standards, controls, samples, anti-serum and hACTH-125I to the test tubes as indicated in the protocol. Shake test tube rack for 30 seconds, vortex tubes thoroughly and incubate at 4°C ± 2° for at least 16 hours.

2. After incubation, locate the bottle of precipitant solution (at room temperature) and mix well with shaking. Add 0.5 mL of this solution to all tubes.

3. Shake the test tube rack for 30 seconds and vortex tubes thoroughly until one homogeneous color is seen in the tube. (If a "parfait" effect is seen in tube, it has not been mixed enough.) Centrifuge at 950-1050 x g for 10 - 15 minutes (for refrigerated centrifuges, a temperature of 6° to 8°C is suggested).

NOTE: For optimum results, tubes should be centrifuged within 15 minutes after the addition of the precipitant solution.

4. Aspirate or decant the supernatant. (If decanting, blot the rim of the test tube on absorbent paper before turning upright.) Count the precipitate remaining in the tubes in a gamma counter. A counting time of at least 2 minutes per tube is suggested.

NOTE: For optimum accuracy, values exceeding 500 pg/mL should be diluted with the zero standard and re-assayed.

C. QUALITY CONTROL

The controls provided with this kit are plasma pools containing different concentrations of ACTH and should routinely be assayed as unknowns. The concentrations of these controls should be plotted daily on a Levey-Jennings type system in order to monitor the performance and reliability of the assay. Quality control parameters, as well as the lot numbers of all reagents, should be recorded for each assay run. For further information, see: DAVID RODBARD: "Statistical Quality Control and Routine Data Processing for Radioimmunoassays and Immunoradiometric Assays." CLIN CHEM 20/10, 1255-1270 (1974).

X. PROTOCOL

<table>
<thead>
<tr>
<th>Tube No.</th>
<th>Description</th>
<th>Diluent Water (mL)</th>
<th>Standard/Sample (mL)</th>
<th>Anti-ACTH (mL)</th>
<th>hACTH-125I (mL)</th>
<th>Precipitant Solution (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,2</td>
<td>Blank</td>
<td>0.1</td>
<td>0.1 (0 pg/mL)</td>
<td>0</td>
<td>0.1</td>
<td>0.5</td>
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<td>3,4</td>
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<td>0.1</td>
<td>0.1</td>
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<tr>
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<tr>
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<tr>
<td>29,30</td>
<td>Sample</td>
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<tr>
<td>31,32</td>
<td>Sample</td>
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<tr>
<td>33,34</td>
<td>Sample</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

IX. FORMULA:

\[
\begin{align*}
\text{%B/Bo} & = \frac{\text{CPM(sample)} - \text{CPM(NSB)}}{\text{CPM(0 pg/mL)} - \text{CPM(NSB)}} \times 100 \\
\text{CPM} & = \text{Average counts of a duplicate} \\
\text{Sample} & = \text{Particular serum or standard being calculated.} \\
\text{NSB} & = \text{Non-specific binding tube (also known as the blank)} \\
0 \text{pg/mL} & = \text{0 tube (also known as Bo or total binding tube)}
\end{align*}
\]

Example: 10 pg/mL calculation:

\[
\begin{align*}
\text{%B/Bo} & = \frac{8836 - 1564}{9493 - 1564} \times 100 \\
& = \frac{7272}{7929} \times 100 \\
& = 92\%
\end{align*}
\]

C. Construct a plot of the percent bound (y-axis) versus the concentration of the ACTH standards (x-axis) starting with the 10 pg/mL point. Either log-logit or semi-log paper may be used. This yields the standard curve.

D. Using the standard curve, determine the ACTH concentrations of each patient sample.

NOTE: Values for samples that bind either higher or lower than the standard curve should not be determined by extrapolation.

XI. SAMPLE ASSAY

These calculations are for example only. The user must construct a standard curve each time the assay is run.

A. Average the counts of all duplicates. Subtract the average non-specific binding counts from the averages obtained above. This yields the corrected counts. Divide these corrected counts by the corrected Bo counts to obtain the percent bound (B/Bo).

B. Formula:

\[
\begin{align*}
\text{%B/Bo} & = \frac{\text{CPM(sample)} - \text{CPM(NSB)}}{\text{CPM(0 pg/mL)} - \text{CPM(NSB)}} \times 100 \\
\text{CPM} & = \text{Average counts of a duplicate} \\
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XII. SAMPLE ASSAY

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<table>
<thead>
<tr>
<th>Tube No.</th>
<th>Description</th>
<th>CTS*</th>
<th>Averaged CTS*</th>
<th>Avg.-Blank CTS*</th>
<th>% Bound</th>
<th>Result (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank (Non-Specific binding)</td>
<td>1621</td>
<td>1508</td>
<td>1564</td>
<td>9475</td>
<td>9511</td>
<td>7929</td>
</tr>
<tr>
<td>Zero Standard (100% binding)</td>
<td>9493</td>
<td>9511</td>
<td>7929</td>
<td>8836</td>
<td>8779</td>
<td>7272</td>
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<td>7984</td>
<td>6420</td>
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<td>9779</td>
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<td>25 pg/mL Standard</td>
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<td>4296</td>
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<td>1394</td>
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<td>828</td>
<td>828</td>
<td>488</td>
<td>2458</td>
<td>828</td>
<td>488</td>
</tr>
<tr>
<td>1000 pg/mL Standard</td>
<td>828</td>
<td>828</td>
<td>488</td>
<td>2458</td>
<td>828</td>
<td>488</td>
</tr>
<tr>
<td>Plasma Sample</td>
<td>6084</td>
<td>6040</td>
<td>4520</td>
<td>6188</td>
<td>6084</td>
<td>4520</td>
</tr>
<tr>
<td>Plasma Sample</td>
<td>6084</td>
<td>6040</td>
<td>4520</td>
<td>6188</td>
<td>6084</td>
<td>4520</td>
</tr>
<tr>
<td>Plasma Sample</td>
<td>6084</td>
<td>6040</td>
<td>4520</td>
<td>6188</td>
<td>6084</td>
<td>4520</td>
</tr>
</tbody>
</table>

* Two minute counts
XII. SAMPLE STANDARD CURVE

NOTE: This curve serves only as an example. Patient sample concentrations must not be derived from it.

XIII. EXPECTED PHYSIOLOGICAL RANGES

(Standardization against W.H.O. 74/555 hACTH preparation) The following normal values were obtained using EDTA plasma samples.

A.M. (8:00 - 9:00) < 10 pg/mL to 100 pg/mL*
P.M. (4:00 - 5:00) < 10 pg/mL to 80 pg/mL*

*Laboratories not using EDTA plasma must establish their own normals.

XIV. PERFORMANCE CHARACTERISTICS

A. This assay is standardized against the W.H.O. 74/555 ACTH preparation.

B. To demonstrate the accuracy of this kit, known amounts of ACTH-39 were added to plasma samples. The following data was obtained:

<table>
<thead>
<tr>
<th>Sample</th>
<th>ACTH Added (pg/mL)</th>
<th>ACTH Expected (pg/mL)</th>
<th>ACTH Obtained (pg/mL)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>12.5</td>
<td>52.5</td>
<td>56</td>
<td>107</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>90</td>
<td>92</td>
<td>102</td>
</tr>
<tr>
<td></td>
<td>125</td>
<td>165</td>
<td>180</td>
<td>109</td>
</tr>
<tr>
<td>B</td>
<td>12.5</td>
<td>25.5</td>
<td>23.5</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>65</td>
<td>61</td>
<td>103</td>
</tr>
<tr>
<td></td>
<td>125</td>
<td>145</td>
<td>145</td>
<td>107</td>
</tr>
</tbody>
</table>

C. Parallelism

Four (4) plasma samples were serially diluted (1:2, 1:4, 1:6, 1:8) with the zero standard and were assayed. The following data was obtained.*

<table>
<thead>
<tr>
<th>Sample</th>
<th>ACTH Added (pg/mL)</th>
<th>ACTH Expected (pg/mL)</th>
<th>ACTH Obtained (pg/mL)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>120</td>
<td>58</td>
<td>39</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>240</td>
<td>232</td>
<td>234</td>
<td>224</td>
</tr>
<tr>
<td>B</td>
<td>190</td>
<td>96</td>
<td>67</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>380</td>
<td>384</td>
<td>402</td>
<td>376</td>
</tr>
<tr>
<td>C</td>
<td>290</td>
<td>140</td>
<td>100</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>580</td>
<td>560</td>
<td>600</td>
<td>560</td>
</tr>
<tr>
<td>D</td>
<td>370</td>
<td>195</td>
<td>135</td>
<td>96</td>
</tr>
</tbody>
</table>

D. Intra-Assay Variation (n=10) | E. Inter-Assay Variation (n=10)

<table>
<thead>
<tr>
<th>Compound</th>
<th>% Cross Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACTH-39</td>
<td>100</td>
</tr>
<tr>
<td>ACTH-24</td>
<td>100</td>
</tr>
<tr>
<td>hβ Lipotropin</td>
<td>0.8</td>
</tr>
<tr>
<td>hα Lipotropin</td>
<td>0.1</td>
</tr>
<tr>
<td>hβ Endorphin</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>hα MSH</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>hβ MSH</td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>

XVI. REFERENCES

5. Besser, G.M., CLIN. ENDOCR. 2:175, (1973)