ACTH [Adrenocorticotropic Hormone] ELISA

REF **7023**

Specific quantitative assay for the determination of adrenocorticotropic hormone in plasma

July 2018

BIOMERICA

I. INTENDED USE

The Biomerica ACTH ELISA is intended for the quantitative determination of ACTH (Adrenocorticotropic Hormone) in human plasma. This assay is intended for *in vitro* diagnostic use.

II. SUMMARY AND EXPLANATION

ACTH (Adrenocorticotropic hormone) or corticotropin is a 39-amino acid peptide hormone (MW=4500) secreted by the pituitary to regulate the production of steroid hormones by the adrenal cortex. ACTH secretion from the anterior pituitary is controlled by both a classical negative feedback control mechanism and CNS-stress mediated control system.[1] Various types of stress or pain perceived in higher levels of the brain modulate secretion of the hypothalamic neurosecretory hormone, corticotropin releasing hormone (CRH), a 41-amino acid peptide. 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. ACTH increases the synthesis and release of all adrenal steroids, aldosterone, cortisol and adrenal androgens. It is the principal modulator of cortisol, the most important glucocorticoid in man. As the cortisol level in blood increases, release of ACTH is inhibited directly at the pituitary level. Through this same mechanism, decreasing cortisol levels lead to elevated ACTH levels. [2,3,4,5]

Biologically active ACTH results from enzymatic cleavage of a large precursor molecule, pro-opiomelanocortin (POMC). This molecule contains within its structure the amino acid sequences of ACTH, Pro-ACTH, ß-melanocyte stimulating hormone, lipotropin, as well as endorphin and the enkephalins. Because the reaction in immunoassays is determined by antigenic structure, not biological function, the usual ACTH RIA reacts with POMC, Pro-ACTH, ACTH and some fragments of the ACTH.[5]

Like other pituitary hormones, ACTH is secreted in a pulsatile manner. These small pulses are superimposed on a characteristic diurnal fluctuation of greater amplitude. In healthy individuals, ACTH reaches a peak in the early morning (6:00 - 8:00 hour) and levels become lowest late in the day and near the beginning of the sleep period. Because of this diurnal rhythm, it is customary to draw plasma ACTH samples between 8:00 and 10:00 hour. However, differentiation of patients with Cushing's disease from normal individuals may be best achieved on samples obtained in the evening (16:00 - 18:00 hour). In Cushing's disease and in ectopic ACTH syndromes, the diurnal pattern of ACTH secretion is generally absent. Stress may also override the diurnal variation.

III. CLINICAL SIGNIFICANCE

Plasma ACTH assays are useful in the differential diagnosis of pituitary Cushing's disease, Addison's disease, autonomous ACTH producing pituitary tumors (e.g. Nelson's syndrome), hypopituitarism with ACTH deficiency and ectopic ACTH syndrome. [5,6,7,8,9,10]

Cushing's syndrome is caused by the effects of excess glucocorticoid actions. All causes of Cushing's syndrome, with the exception of glucocorticoid medication, are associated with increased 24-hour urinary cortisol. The most common cause of Cushing's syndrome is bilateral adrenal hyperplasia, due to pituitary ACTH hypersecretion (Cushing's disease) from a pituitary adenoma or corticotroph hyperplasia.[5,6,7,8,9,10] Laboratory diagnosis of Cushing's disease is supported by the following: (1) suppression of plasma ACTH and cortisol concentrations, by high-dose (2.0 mg q 6h x 8) dexamethasone administration, (2) absence of ACTH and cortisol suppression with low-dose (0.5 mg q 6h x 8 or 1 mg given at 23:30 hour) dexamethasone, (3) larger than normal response to metyrapone (Metopirone) stimulation and normal or elevated plasma ACTH levels.[4]

When Cushing's syndrome is caused by primary adrenal Hbnormality (adenoma or carcinoma), the adrenal gland acts independently of ACTH and pituitary ACTH secretion is suppressed. [5,6,7,8,9,10] Hence, there is no response to dexamethasone suppression or metyrapone stimulation. This type of Cushing's syndrome is characterized by very low or undetectable levels of ACTH.

Therefore, measurement of plasma ACTH is helpful in differential diagnosis of pituitary Cushing's syndrome. In patients with adrenal tumors, ACTH levels are low. High levels of ACTH are seen in patients with ectopic ACTH syndrome. Patients with bilateral adrenal hyperplasia will have ACTH levels inappropriately elevated for their degree of hypercortisolism, which should suppress ACTH. However, in most cases the ACTH concentration will be within the normal range.

Adrenocortical insufficiency or inadequate cortisol production can be due to destruction of the adrenal cortex or to abnormalities of the pituitary or hypothalamus, which result in inadequate ACTH production of release. [5,6,7,8,9,10] Primary adrenocortical insufficiency, Addison's disease, is characterized by markedly elevated plasma ACTH levels and adrenal unresponsiveness to stimulation with exogenous ACTH. Hypopituitarism with ACTH deficiency, which is secondary adrenocortical insufficiency, is characterized by low plasma ACTH and cortisol concentrations, and a subnormal, but usually distinct adrenal response to stimulation with synthetic ACTH (Cortrosyn®). If hypoglycemic stress or metyrapone stimulation is required for diagnosis, ACTH and cortisol responses are less than normal.

Aggressive and invasive ACTH producing pituitary tumors occurring before or following bilateral adrenalectomy for Cushing's disease (Nelson's syndrome) are characterized by the development of Addisonian pigmentation, often in an adrenalectomized patient who is taking adequate glucocorticoid replacement therapy. In these patients, plasma ACTH levels are markedly elevated and do not respond well to dexamethasone suppression.

IV. PRINCIPLE OF THE TEST

The Biomerica ACTH Immunoassay is a two-site ELISA [Enzyme-Linked ImmunoSorbent Assay] for the measurement of the biologically active 39 amino acid chain of ACTH. A goat polyclonal antibody to human ACTH, purified by affinity chromatography, and a mouse monoclonal antibody to human 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.

> Streptavidin Well - Biotinylated Anti-ACTH (34-39) -- ACTH -- HRP conjugated Anti-ACTH (1-24)

In this assay, calibrators, controls, or patient 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 and the enzyme bound to the solid phase is incubated with the substrate, tetramethylbenzidine (TMB). An acidic stopping solution is then added to stop the reaction and converts the color to yellow. The intensity of the yellow color is directly proportional to the concentration of ACTH in the sample. A dose response curve of absorbance unit vs. concentration is generated using results obtained from the calibrators. Concentrations of ACTH present in the controls and patient samples are determined directly from this curve.

V. KIT COMPONENTS

Kit Components	Description	Quantity
RGT 1 = Reagent 1	Biotinylated ACTH Antibody [affinity purified goat anti human ACTH]	1 x 2.7 mL
RGT 2 = Reagent 2	Peroxidase (Enzyme) labeled ACTH Antibody [mouse monoclonal anti human ACTH]	1 x 2.7 mL
RGT A = *ELISA* Reagent A	ELISA Wash Concentrate [Saline with surfactant]	1 x 30 mL
***RGT B = ELISA* Reagent B**	TMB Substrate [tetramethylbenzidine]	1 x 15.5 mL
SOLN = Stopping solution	ELISA Stop Solution [1 N sulfuric acid]	1 x 20 mL
PLA = Microplate	One holder with Streptavidin Coated Strips.	12 x 8-well strips
CAL = Calibrators A: 0 pg/mL B: C: Refer to vial D: Labels for exact E: concentrations F:	Lyophilized [except zero calibrator] synthetic h-ACTH. Zero calibrator [BSA/equine serum solution] is in liquid form, ready to use. All other calibrators consist of synthetic h-ACTH (1-39) in BSA/equine serum solution	1 x 4 mL for the zero calibrator 1 x 2 mL for all other calibrators
CTRL = Controls 1 & 2 Refer to vial labels for exact ranges	Lyophilized. 2 Levels. Synthetic h-ACTH (1-39) in BSA/equine serum solution.	1 x 2 mL per level

MATERIAL AND EQUIPMENT REQUIRED BUT NOT PROVIDED
- Microplate reader.
- Microplate washer [if washer is unavailable, manual washing may be acceptable].
- Precision Pipettors to deliver 25, 200, 100 and 150 µL.

- *(Optional):* A multi-channel dispenser or a repeating dispenser for 25, 100 and 150 µL.
- Microplate Shakers: Biomerica has found for shaker diameters indicated below, the Streptavidin kits will maintain optimal performance response at the following speed settings:

Microplate Shakers	Shaking diameter	Speed setting
Orbital	3 mm (0.1118 in)	600 ± 10 rpm
	19 mm (0.75 in)	170 ± 10 rpm
Linear	25 mm (0.98 in)	170 ± 10 rpm

VI.   WARNINGS AND PRECAUTIONS FOR USERS

Although the reagents provided in this kit has been specifically designed to contain no human blood components, the human patient samples, which might be positive for HBsAg, HBcAg or HIV antibodies, must be treated as potentially infectious biohazard. Common precautions in handling should be exercised, as applied to any untested patient sample.

Stopping Solution consists of 1 N Sulfuric Acid. This is a strong acid. Although diluted, it still must be handled with care. It can cause burns and should be handled with gloves and eye protection, with appropriate protective clothing. Any spill should be wiped immediately with copious quantities of water. Do not breathe vapor and avoid inhalation.

If turbidity is observed in any reagent, do not perform assay and please contact your supplier.

Various types of shakers with different specifications are commercially available. In the event that the microplate shaker does not fall within the specified range above, each laboratory is encouraged to set their own optimal range.

VII.   SAMPLE COLLECTION AND STORAGE

The determination of ACTH should be performed on EDTA plasma. To assay the specimen in duplicate, 400 µL of EDTA plasma is required. Collect whole blood in a lavender [EDTA] tube. The plasma should be promptly separated, preferably in a refrigerated centrifuge, and stored at -20°C or lower. EDTA plasma samples may be stored up to 8 hours at 2-8°C. EDTA plasma samples frozen at -20°C are stable for up to 4 months.

VIII.   REAGENT PREPARATION AND STORAGE

Store all kit components at 2-8° C.
1.   All reagents except the non-zero calibrators, kit controls and the Wash Concentrate are ready-to-use. Store all reagents at 2-8° C.
2.   For each of the non-zero calibrators (Calibrator B through F) and kit controls 1 and 2, 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 when handled as recommended in "Procedural Notes" section.
3.   *ELISA Reagent A*: Wash Concentrate: Mix contents of wash concentrate thoroughly. If precipitate is present in the Wash Concentrate due to storage at lower temperature such as 4°C, dissolve by placing the vial in a 37°C water bath or oven with swirling or stirring. Add wash concentrate (30 mL) to 570 mL of distilled or deionized water and mix. The diluted working wash solution is stable for 90 days when stored at room temperature.

XI.   ASSAY PROCEDURE

1.   Place sufficient **Streptavidin Coated Strips** in a holder to run all six (6) ACTH calibrators, A - F of the ACTH CALIBRATORS [Exact concentration is stated on the vial label], Quality Control Plasma and patient samples. At a minimum, designate two wells to serve as "blanks". Refer to Step 9 for final plate reading.
2.   Pipet **200 µL** of calibrators, controls, and samples into the designated or mapped well. *Freeze (-20°C) the remaining calibrators and controls as soon as possible after use.*
3.   Add or dispense **25 µL** of Reagent 1 (Biotinylated Antibody) into each of the wells, which contain the calibrators, controls, and samples.
4.   Add or dispense **25 µL** of Reagent 2 (Enzyme Labeled Antibody) into each of the same wells.
      Cover the microplate(s) with aluminum foil or a tray to avoid exposure to light, and place it on a **shaker** set at recommended settings (see section **V**) for **4 hours ± 30 minutes** at room temperature (22°-28°C).
5.   First aspirate the fluid completely and then wash/aspirate each well five (5) times with the Working Wash Solution (prepared from Reagent A), using an automatic microplate washer. The wash solution volume should be set to dispense 0.35 mL into each well.
6.   Add or dispense **150 µL** of the *ELISA Reagent B* (TMB Substrate) into each of the wells, except the blank wells.
7.   With appropriate cover to avoid light exposure, place the microplate(s) on a **shaker** set at recommended settings (see section **V**) for **30 ± 5 minutes** at room temperature (22°-28°C).
8.   Add or dispense **100 µL** of the Stopping Solution into each of the wells, except the blank wells. Mix gently.
9.   Prior to reading, ensure both "blank wells" as mentioned in Step 1 are filled with 250 µL of distilled or deionized water. Blank the plate reader according to the manufacturer's instructions by using the blank wells.***** Read the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to **450 nm**. **Read** the plate **again** with the reader set to **405 nm** also against distilled or deionized water**.**
      * If due to technical reasons the ELISA plate reader cannot be adjusted to zero using "blank," subtract the "blank," absorbance value from all other absorbance values to obtain results.
      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, patient 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.
10.   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.

PROCEDURAL NOTES
- ACTH 1-39 is a very labile molecule. Set up the assay immediately upon the reconstitution or the thawing of all calibrators, controls, and patient samples.
- It is recommended that all calibrators, controls, and patient samples are assayed in duplicate. The average absorbance units of duplicate sets should then be used for reduction of data and the calculation of results.
- The samples should be pipetted into the well with minimum amount of air-bubble. To achieve this, "reverse pipet" described in the package insert of the manufacturers of Pipettors is recommended.
- Patient samples with values greater than the highest calibrator (Calibrator F), which is approximately 500 pg/mL (see exact concentration on vial label), may be diluted with Calibrator A (Zero Calibrator) and reassayed. Multiply the result by the dilution factor.
- Reagents from different lot numbers must not be interchanged.
- If preferred, mix in equal volumes, in sufficient quantities for the assay, Reagent 1 (Biotinylated Antibody) and Reagent 2 (Enzyme Labeled Antibody) in a clean amber bottle. Then use 50 µL of the mixed antibody into each well. This alternative method should replace Step (3) and (4), to be followed with the incubation with orbital shaker.
- When mixing avoid splashing of reagents from wells. This will affect assay precision and accuracy.

X.   CALCULATION OF RESULTS

Manual Method
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 three calibrators with the highest concentrations, i.e. Calibrators 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 Y-axis.
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. 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.

Automated Method:
Computer programs using cubic spline or 4 PL [4 Parameter Logistics] or Point-to-Point can generally give a good fit.

Sample Data *at 450 nm* [raw A.U. readout against distilled or deionized water]

Microplate Well	1st Reading Absorbance	2nd Reading Absorbance	Average Absorbance	ACTH pg/mL	ACTH pg/mL–

	Unit	Unit	Unit		Result to report
Calibrator A	0.020	0.018	0.019		0
Calibrator B	0.077	0.074	0.076		5
Calibrator C	0.221	0.229	0.225		18
Calibrator D	0.624	0.692	0.685		55
Calibrator E	1.802	1.934	1.868		165
Control 1	0.417	0.398	0.408	33.5	33.5
Control 2	2.868	2.774	2.821	> 150	*
Patient Sample 1	0.072	0.078	0.075	4.9	4.9
Patient Sample 2	0.185	0.177	0.181	14.0	14.0
Patient Sample 3	0.495	.491	.493	40.8	40.8
Patient Sample 4	2.090	2.122	2.106	> 150	*

* Because the concentration readout is > 150 pg/mL, it is recommended to use the data obtained at 405 nm as shown in **Sample Data** *at 405 nm* in the table below.

Sample Data *at 405 nm* [raw A.U. readout against distilled or deionized water]

Microplate Well	1st Reading Absorbance Unit	2nd Reading Absorbance Unit	Average Absorbance Unit	ACTH pg/mL	ACTH pg/mL– Result to report
Calibrator A	0.011	0.008	0.0095		0
Calibrator D	0.032	0.032	0.032		55
Calibrator E	0.074	0.081	0.078		165
Calibrator F	1.838	1.817	1.828		500
Control 1	0.138	0.132	0.135	< 150	¶
Control 2	0.921	0.894	0.908	256	256
Patient Sample 1	0.030	0.032	0.031	< 150	¶
Patient Sample 2	0.068	0.062	0.065	< 150	¶
Patient Sample 3	0.165	0.159	0.162	< 150	¶
Patient Sample 4	0.663	.677	0.670	188	188

¶ For samples with readout < 150 pg/mL, it is recommended to use the data obtained at 450 nm as shown in **Sample Data** *at 450 nm* in the table above. This practice should give the results with optimum sensitivity of the assay.

NOTE: The data presented are for illustration purposes only and must not be used in place of data generated at the time of the assay.

XI. QUALITY CONTROL

Control plasma or plasma pools should be analyzed with each run of calibrators and patient 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.

XII. LIMITATIONS OF THE PROCEDURE

The Biomerica 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.

Supplements containing high biotin levels such as those marketed for hair, skin, and nail benefits, may contain interfering biotin amounts. Biotin levels higher than the recommended daily allowance may cause interference with the assay. Therefore, it is important to communicate with health care providers and patients about biotin intake when collecting samples to prevent incorrect test results. Results show that the highest concentration at which no significant interference was observed is 2 ng/mL of D-Biotin.

Samples from patients routinely exposed to animal or animal serum products may contain heterophilic antibodies causing atypical results. This assay has been formulated to mitigate the risk of this type of interference. However, potential interactions between rare sera and test components can occur.

XIII. EXPECTED VALUES

ACTH levels were measured in one hundred and thirty four (134) apparently normal individuals in the U.S. with the Biomerica ACTH ELISA. The values obtained ranged from 7.0 to 63 pg/mL. Based on statistical tests on skewness and kurtosis, the population, when transformed logarithmically, follows the normal or Gaussian distribution. The geometric mean $\pm$ 2 standard deviations of the mean were calculated to be 6.17 to 58.2 pg/mL.

XIV. PERFORMANCE CHARACTERISTICS

Accuracy
Three hundred (300) patient samples, with ACTH values ranging from 1.0 to 640 pg/mL were assayed by the previous Biomerica ACTH kit and the updated Biomerica ACTH kit ELISA. Linear regression analysis gives the following statistics:

$$\text{Biomerica ELISA} = 1.02 \text{ ELISA Kit} - 1.58 \text{ pg/mL}$$
$$r = 0.995 \quad N = 300$$

Sensitivity
The sensitivity, or minimum detection limit, of this assay is defined as the smallest single value, which can be distinguished from zero at the 95% confidence limit. The Biomerica ACTH ELISA has a calculated sensitivity of 0.22 pg/mL.

Precision and Reproducibility
The precision (intra-assay variation) of the Biomerica ACTH ELISA Test was calculated from 25 replicate determinations on each of the two samples.

Intra-Assay Variation

Sample	Mean Value (pg/mL)	N	Coefficient of variation %
A	42.2	25	6.71
B	269.9	25	2.27

The total precision (inter-assay variation) of the Biomerica ACTH ELISA Test was calculated from data on two samples obtained in 21 different assays, by three technicians on three different lots of reagents, over a four-week period.

Inter-Assay Variation

Sample	Mean Value (pg/mL)	N	Coefficient of variation %
A	42.3	21	7.1
B	287.8	21	6.9

Specificity and Cross-Reactivity
Cross-reactivity in the ACTH was studied by the addition of various materials to an ACTH standard. The results are as follows:

Cross-reactant	Concentration of Cross-reactant	ACTH without Cross-reactant [pg/ml]	ACTH With Cross-reactant [pg/ml]	Change in ACTH [pg/ml]	% Cross-reactivity
ACTH (1-24)	100 000 pg/ml	62.9	0.8	-62.1	-0.06 %
	10 000 pg/ml	62.9	5.05	-57.85	-0.58 %
	1000 pg/ml	62.9	28.6	-34.3	-3.43 %
	200 pg/ml	62.9	43.4	-19.5	-9.75 %
ACTH (18-39)	5000 pg/ml	61.2	2	-59.2	-1.2 %
	2000 pg/ml	61.2	13.6	-47.6	-2.4 %
	500 pg/ml	61.2	24.3	-36.9	-7.4 %
a-MSH	100 000 pg/ml	88.1	65.7	-22.4	-0.02 %
	10 000 pg/ml	88.1	69.1	-19	-0.19 %
	1000 pg/ml	88.1	70.7	-17.4	-1.7 %
	200 pg/ml	88.1	74.8	-13.3	-6.7 %
b-ENDORPHIN	100 000 pg/ml	73.8	60.5	-13.3	-0.01 %
	50 000 pg/ml	73.8	56.9	-16.9	-0.03 %

Recovery
Various amounts of ACTH were added to four different patient plasma to determine the recovery. The results are described in the following table:

Plasma Sample	Endogenous ACTH (pg/ml)	ACTH added (pg/ml)	Expected Value (pg/ml)	Measured Value (pg/ml)	Recovery (%)
A	13.3	50.0	63.3	62.4	99 %
		100.0	113.5	116	102 %
B	17.7	50.0	67.7	62.1	92 %
		100.0	117.7	121.7	103 %
C	14.8	50.0	64.8	64.2	99 %
		100.0	114.8	114.2	99 %
D	27.1	50.0	77.1	67.4	87 %
		100.0	127.1	119	94 %

Kinetic Effect of the Assay
To determine whether there is any systematic kinetic effect between the beginning of the run and the end of the run, three spiked patient pools, selected to represent a good cross section of the ACTH concentration, were placed in sequence throughout the run of one microplate or 96 wells [with twelve 8-well strips].

**Linearity of Patient Sample Dilutions: Parallelism**

Five patient plasma samples were diluted with Calibrator A (Zero Calibrator). Results in pg/mL are shown below:

Sample	Dilution	Expected pg/mL	Observed pg/mL	% Observed ÷ Expected
A	Undiluted	-	288	-
	1:2	144	150	104%
	1:4	72	70.9	98%
	1:8	36	35.7	99%
B	Undiluted	-	468	-
	1:2	234	278	119%
	1:4	117	135	115%
	1:8	58.5	65.5	112%
C	Undiluted	-	270	-
	1:2	135	146	108%
	1:4	67.5	68	101%
	1:8	33.75	33.5	99%
D	Undiluted	-	336	-
	1:2	168	149	89%
	1:4	84	83	99%
	1:8	42	47	112%
E	Undiluted	-	452	-
	1:2	226	268	119%
	1:4	113	126	112%
	1:8	56.5	68.9	122%

XV. REFERENCES:

1. Ryan, WG: Endocrine Disorders – A Pathophysiologic Approach, 2nd Edition Year Book Medical Publishers, Inc. 1980.
2. Watts, N.B., J.H. Keffer: Practical Endocrine Diagnosis, Third Edition, Lea and Febioer, 1982.
3. Ganong, WF. Alpert LC, Lee TC: ACTH and the Regulation of Adrenocorticol Secretion, N. Engl. J. Med. 290 : 1006, 1974.
4. Tepperman, J: Metabolic and Endocrine Physiology, 4th Edition, Year Book Medical Publishers, Inc., 1981.
5. Odell, W.D., R. Horton, M.R. Pandian, J. Wong: The Use of ACTH and Cortisol Assays in the Diagnosis of Endocrine Disorders. Nichols Institute Publication, 1989.
6. Radioimmunoassay Manual, Edited by A.L. Nichols and J.C. Nelson, 4th Edition Nichols Institute, 1977.
7. Gold, E.M.: The Cushing's Syndromes: Changing Views of Diagnosis and Treatment. Ann Intern. Med. 90:829, 1979.
8. Plasma Cortisol, RIA for Physicians, Edited by J.C. Travis, 1:8, Scientific Newsletter, Inc. 1976.
9. Krieger, D.T.: Physiopathology of Cusihing's Disease, Endocrine Review 4:22-43, 1983.
10. Krieger, D.T., A.S. Liotta, T. Suda, A Goodgold, and E. Condon: Human Plasma Immunoreactive Lipotropin and Adrenocorticotropin in Normal Subjects and in Patients with Pituitary-Adrenal Disease, J. Clin. Endocrinol Metab. 48:566-571, 1979.

XVI. SYMBOLS

〡	Storage Temperature
LOT	Lot Code
⧖	Expiration
▰	Manufacturer
EC REP	Authorized Representative
⚠	Caution, see instructions
IVD	For in vitro diagnostic use
REF	Catalog No.

XVII. ORDERING INFORMATION

▰ BIOMERICA, INC.

Contact:

2°C / 8°C

IVD

CE

Distributed By:
IBL-America, Inc.
8201 Central Ave NE, Suite P
Minneapolis, MN 55432, USA
info@ibl-america.com
(888) 523 1246

EC REP

according to IVDD 98/79/ EC
MDSS GmbH
Schiffgraben 41
D-30175 Hannover
Germany

67023-13_eng.doc

July 2018