ACTH-Irma

KIR0061

History

Resume of change :

Previous Version : 180914/1	**Current Version :** 191227/1
II. GENERAL INFORMATION **For technical assistance or ordering information contact :** **Tel : +32 (0)10 84.99.11 Fax : +32 (0)10 84.99.91**	***II. GENERAL INFORMATION*** **For technical assistance or ordering information contact :** **Tel : +32 (0)10 84.99.11 Fax : +32 (0)10 84.99.91** **For technical assistance or ordering information in the United States contact :** **Immuno-Biological Laboratories, Inc. (IBL-America)** **Tel : 1-888-523-1246 Fax : 1-763-780-2988 Email : info@ibl-america.com**
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Read entire protocol before use.

ACTH-IRMA

I. INTENDED USE

Immunoradiometric assay kit for the *in vitro* quantitative measurement of human Adrenocorticotropic Hormone (ACTH) in EDTA plasma.
For Research Use Only. Not for Use in Diagnostic Procedures.

II. GENERAL INFORMATION

A. Proprietary name : DIAsource ACTH-IRMA Kit

B. Catalog number : KIR0061: 96 tests

C. Manufactured by : DIAsource ImmunoAssays S.A.
Rue du Bosquet, 2, B-1348 Louvain-la-Neuve, Belgium.

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III. BACKGROUND

Biological Activity

Adrenocorticotropic hormone (ACTH or corticotrophin) is a polypeptide hormone synthesised (from POMC, pro-opiomelanocortin) and secreted from corticotropes in the anterior lobe of the pituitary gland in response to the hormone corticotrophin-releasing hormone (CRH) released by the hypothalamus. It consists of 39 amino acids with a molecular weight of 4540 Da.
ACTH regulates steroid synthesis by the adrenal cortex. ACTH stimulates the secretion of cortisol from the adrenal glands. Cortisol and other glucocorticoids increase glucose production, inhibit protein synthesis and increase protein breakdown, stimulate lipolysis, and affect immunological and inflammatory responses. Cortisol induces thymus involution which is a decline in normal thymus function that in part accounts for its ability to decrease immune system response. Glucocorticoids help maintain blood pressure and from an essential component of the body's response to stress. ACTH secretion is regulated by corticotrophin- releasing hormone (CRH) and vasopressin (ADH). Cortisol feeds back to the pituitary and hypothalamus to suppress levels of ACTH and CRH. Under basal (non-stress) conditions, cortisol is secreted with a pronounced circadian rhythm, with higher levels early in the morning and low levels late in the evening. Under stressful conditions, the circadian variation is blunted.
Non-adrenal gland mediated effects : ACTH stimulates the release of MSH (melanotropic hormone) and GH (growth hormone), increases lipolysis in fat cells (adipocytes), and induces neurological effects (such as stretching and yawning). Much of this is related to its origin from POMC. Lipolysis by ACTH is much weaker than that of lipotropin (LPH). ACTH is a precursor of α-MSH.
In the adrenal cortex, there are two types of ACTH receptors, one with a KD = 1nM, but only about 60 per cell while the other has a KD = 300nM, but with about 600,000 per cell. The presence of high and low affinity receptors for ACTH means that tissues are sensitive not only to the presence of ACTH, but to its concentration.

IV. PRINCIPLES OF THE METHOD

The DIAsource ACTH-IRMA is a two-step immunoradiometric assay based on coated-tube separation. It allows the determination of intact human adrenocorticotropic hormone (ACTH) in EDTA plasma. Monoclonal antibodies specific to the 1-24 ACTH fragment (N-terminal fragment) are attached to the lower and inner surface of the plastic tubes. Calibrators or samples are added to the tubes. After 2 hour incubation, washing removes the occasional excess of antigen, mid-regional and C-terminal fragments.
^{125}I labelled polyclonal antibodies specific to the 24-39 ACTH fragment (C-terminal fragment) are added. After 1 hour incubation and washing the remaining radioactivity bound to the tube reflects the intact ACTH concentration.

V. REAGENTS PROVIDED

Reagents	Quantity 96 tests	Colour Code	Reconstitution
Tubes coated with anti ACTH (monoclonal antibodies)	2 x 48	red	**Ready** for use
Ab | **125I** | **CONC** Anti-ACTH-^{125}I (polyclonal antibodies) in Phosphate Buffer with bovine albumin and sodium azide (<0.1%)	1 vial 0.8 ml 1020 kBq	red	**Dilute** 21x with Tracer Buffer (see section VII. C)
TRACER | **BUF** Tracer Buffer: Borate Buffer with sheep serum, EDTA and azide (<0.1%)	1 vial 11 ml	black	**Ready** for use
CAL | **0** Zero Calibrator in human plasma with thymol and benzamidin	1 vial lyophil.	yellow	**Add** 5 ml reconstitution solution
CAL | **N** Calibrators 1-6 in human plasma with thymol and benzamidin (see exact values on vial labels)	6 vials lyophil.	yellow	**Add** 1 ml reconstitution solution
REC | **SOLN** Reconstitution solution : Borate Buffer with EDTA and sodium azide (< 0.1%)	1 vial 15 ml	blue	**Ready** for use
INC | **BUF** Incubation Buffer: Phosphate Buffer with bovine albumin and azide (<0.1%)	1 vial 6 ml	black	**Ready** for use
WASH | **SOLN** | **CONC** Wash solution (Tween 20-NaCl)	1 vial 40 ml	brown	**Dilute** 20x with distilled water (use a magnetic stirrer).
CONTROL | **N** Controls 1 and 2 in human plasma with thymol	2 vials lyophil.	silver	**Add** 1 ml reconstitution solution

Note: 1. Use the zero calibrator for sera dilutions.
2. 1 pg of the calibrator preparation is equivalent to 1 pg of NIBSC 74/555

VI. SUPPLIES NOT PROVIDED

The following material is required but not provided in the kit:
1. Distilled water
2. Pipettes for delivery of: 50 µl, 100 µl, 200 µl, 1 ml and 3 ml. (the use of accurate pipettes with disposable plastic tips is recommended)
3. Vortex mixer
4. Magnetic stirrer
5. Tube shaker (400 rpm)
6. 5 ml automatic syringe (Cornwall type) for washing
7. Aspiration system (optional).
8. Any gamma counter capable of measuring ^{125}I may be used (minimal yield 70%).

VII. REAGENT PREPARATION

A. **Calibrators** : Reconstitute the zero calibrator with 5 ml reconstitution solution and the other calibrators with 1 ml reconstitution solution.
B. **Controls** : Reconstitute the controls with 1 ml reconstitution solution.
C. **Tracer :** Prepare an adequate volume of Tracer solution by adding 50 µl of Anti-ACTH-^{125}I to 1 ml of Tracer Buffer. Use a vortex to homogenize.
D. **Working Wash solution** : Prepare an adequate volume of Working Wash solution by adding 19 volumes of distilled water to 1 volume of Wash Solution (20x). Use a magnetic stirrer to homogenize. Discard unused Working Wash solution at the end of the day.

VIII. STORAGE AND EXPIRATION DATING OF REAGENTS

- Before opening or reconstitution, all kit components are stable until the expiry date, indicated on the label, if kept at 2 to 8°C.
- The calibrators and controls are very unstable, use them immediately after reconstitution, freeze immediately in aliquots and keep them at –20°C for maximum **7 weeks**. Avoid subsequent freeze-thaw cycles.
- Freshly prepared Working Wash solution should be used on the same day.
- After its first use, tracer is stable until expiry date, if kept in the original well-closed vial at -20°C.
- Alterations in physical appearance of kit reagents may indicate instability or deterioration.

IX. SPECIMEN COLLECTION AND PREPARATION

- EDTA plasma should be used and the usual precautions for venipuncture should be observed.
- Specimens should be collected and be placed on ice immediately or drawn into previously chilled tubes. Immediately separate in a refrigerated centrifuge (2-8°C). If not assayed immediately (within one hour), remove the plasma supernatant to the appropriately labelled plastic storage vessel, and freeze at -70°C or colder for up to 45 days.
- Specimens may not remain stable when stored at -20°C.
- Do not use hemolyzed or lipemic specimens.

X. PROCEDURE

A. Handling notes

Do not use the kit or components beyond expiry date. Do not mix materials from different kit lots. Bring all the reagents to room temperature prior to use.
Thoroughly mix all reagents and samples by gentle agitation or swirling. In order to avoid cross-contamination, use a clean disposable pipette tip for the addition of each reagent and sample.
High precision pipettes or automated pipetting equipment will improve the precision. Respect the incubation times.
Prepare a calibration curve for each run, do not use data from previous runs.

B. Procedure

1. Label coated tubes in duplicate for each calibrator, control and sample. For determination of total counts, label 2 normal tubes.
2. Briefly vortex calibrators, controls and samples and dispense 200 µl of each into the respective tubes.
3. Dispense 50 µl Incubation Buffer in each tube except those for total counts.
4. Shake the rack containing the tubes gently by hand to liberate any trapped air bubbles.
5. Incubate for 2 hour at room temperature on a tube shaker (400 rpm).
6. Aspirate the content of each tube (except total counts). Be sure that the plastic tip of the aspirator reaches the bottom of the coated tube in order to remove all the liquid.
7. Wash the tubes with 2 ml Wash Solution (except total counts). Avoid foaming during the addition of the Working Wash Solution.
8. Aspirate the content of each tube (except total counts).
9. Wash again the tubes with 2 ml Wash Solution (except total counts) and aspirate.
10. After the last washing, let the tubes standing upright for two minutes and aspirate the remaining drop of liquid.
11. Dispense 100 µl of anti-ACTH-^{125}I tracer into each tube, including the uncoated tubes for total counts.

12. Shake the rack containing the tubes gently by hand to liberate any trapped air bubbles.
13. Incubate for 1 hour at room temperature on a tube shaker (400 rpm).
14. Aspirate the content of each tube (except total counts). Be sure that the plastic tip of the aspirator reaches the bottom of the coated-tube in order to remove all the liquid.
15. Wash the tubes with 2 ml Wash Solution (except total counts). Avoid foaming during the addition of the Working Wash Solution.
16. Aspirate the content of each tube (except total counts).
17. Wash again the tubes with 2 ml Wash Solution (except total counts) and aspirate.
18. After the last washing, let the tubes standing upright for two minutes and aspirate the remaining drop of liquid.
19. Count the tubes in a gamma counter for 60 seconds.

XI. CALCULATION OF RESULTS

1. Calculate the mean of duplicate determinations.
2. On semi-logarithmic or linear graph paper plot the c.p.m. (ordinate) for each calibrator against the corresponding concentration of ACTH (abscissa) and draw a calibration curve through the calibrator points, reject the obvious outliers.
3. Read the concentration for each control and sample by interpolation on the calibration curve.
4. Computer assisted data reduction will simplify these calculations. If automatic result processing is used, a 4-parameter logistic function curve fitting is recommended.

XII. TYPICAL DATA

The following data are for illustration only and should never be used instead of the real time calibration curve.

ACTH-IRMA		cpm	B/T (%)
Total count		273476	100
Calibrator	0.0 pg/ml	418	0.2
	9.6 pg/ml	1580	0.6
	31.2 pg/ml	3836	1.4
	97.2 pg/ml	10839	4.0
	295.4 pg/ml	27691	10.1
	1006.4 pg/ml	64156	23.5
	1931.9 pg/ml	93486	34.2

XIII. PERFORMANCE

A. Detection Limit

Twenty zero calibrators were assayed along with a set of other calibrators. The detection limit, defined as the apparent concentration three standard deviations above the average counts at zero binding, was 1.16 pg/ml.

B. Specificity

Possible interfering peptides were added to a low and to a high ACTH level plasma EDTA. The apparent ACTH response was measured.

Added analyte to a low ACTH level EDTA plasma		Observed ACTH level (pg/ml)	Added analyte to a medium ACTH level EDTA plasma		Observed ACTH level (pg/ml)
Nothing		0.9	Nothing		34.5
ACTH 1-17 fragment	100000 pg/ml	0.8	ACTH 1-17 fragment	100000 pg/ml	25.0
ACTH 18-39 fragment	100000 pg/ml	7.1	ACTH 18-39 fragment	100000 pg/ml	33.5
Rat ACTH	1000 pg/ml	364.7	Rat ACTH	1000 pg/ml	450.0
αMSH	100000 pg/ml	1.8	αMSH	100000 pg/ml	31.6
βMSH	100000 pg/ml	0.9	βMSH	100000 pg/ml	33.0
βEndorphin	100000 pg/ml	1.1	βEndorphin	100000 pg/ml	32.8

This demonstrates that the ACTH-IRMA does not cross react with ACTH fragments, αMSH, βMSH and βEndorphin but cross react with Rat ACTH at 39%.

C. Precision

Serum	N	<X> ± SD (pg/ml)	CV (%)	Serum	N	<X> ± SD (pg/ml)	CV (%)
A	19	17.5 ± 1.1	6.4	D	15	29.6 ± 1.4	4.8
B	20	69.3 ± 2.1	3.0	E	15	121.9 ± 7.5	6.2
C	20	386.7 ± 15.1	3.9				

SD : Standard Deviation; CV: Coefficient of variation

D. Accuracy

RECOVERY TEST

Sample	Added ACTH (pg/ml)	Recovered ACTH (pg/ml)	Recovery (%)
1	1100.0	1128.0	102.5
	550.0	552.5	100.5
	275.0	273.8	99.6
	137.5	149.0	108.4

DILUTION TEST

Sample	Dilution	Theoretical Concent. (pg/ml)	Measured Concent. (pg/ml)
1	1/1	-	499.4
	1/2	249.7	226.2
	1/4	124.8	111.0
	1/8	62.4	57.5
	1/16	31.2	27.8
	1/32	15.6	16.1
	1/64	7.8	7.4
2	1/1	-	348.4
	1/2	174.2	167.5
	1/4	87.1	89.9
	1/8	43.6	40.0
	1/16	21.8	20.6
	1/32	10.9	10.4
	1/64	5.4	5.7

Samples were diluted with zero calibrator.

E. Time Delay

As shown hereafter, assay results remain accurate even when a sample is dispensed 30 minutes after the calibrator has been added to coated tubes.

	0'	10'	20'	30'
C1	546.1	563.9	563.4	568.9
C2	86.6	88.7	89.0	85.9
C3	44.4	44.4	42.9	43.2
C4	122.4	123.0	122.6	121.0

F. Hook effect

A plasma EDTA sample with an ACTH concentration of 69000 pg/ml gives a signal above the highest calibrator concentration.

XIV. LIMITATIONS

- Specimens who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits which employ mouse monoclonal antibodies.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays.
  Specimens routinely exposed to animals or animal serum products can be prone to this interference and anomalous values may be observed in case of the presence of heterophelic antibodies.

XV. INTERNAL QUALITY CONTROL

- If the results obtained for Control 1 and/or Control 2 are not within the range specified on the vial label, the results cannot be used unless a

satisfactory explanation for the discrepancy has been given.
- If desirable, each laboratory can make its own pools of control samples, which should be kept frozen in aliquots.
- Acceptance criteria for the difference between the duplicate results of the samples should rely on Good Laboratory Practises

XVI. REFERENCE INTERVALS

The values provided below are given only for guidance; each laboratory should establish its own normal range of values.
The range of ACTH levels in 47 normal specimens, expressed as 5% to 95% percentiles, was 9.6 to 49.7 pg/ml.

XVII. PRECAUTIONS AND WARNINGS

Safety
For *research* use only.
This kit contains ^{125}I (half-life: 60 days), emitting ionizing X (28 keV) and γ (35.5 keV) radiations.
This radioactive product can be transferred to and used only by authorized persons; purchase, storage, use and exchange of radioactive products are subject to the legislation of the end user's country. In no case the product must be administered to humans or animals.
All radioactive handling should be executed in a designated area, away from regular passage. A logbook for receipt and storage of radioactive materials must be kept in the lab. Laboratory equipment and glassware, which could be contaminated with radioactive substances, should be segregated to prevent cross contamination of different radioisotopes.
Any radioactive spills must be cleaned immediately in accordance with the radiation safety procedures. The radioactive waste must be disposed of following the local regulations and guidelines of the authorities holding jurisdiction over the laboratory. Adherence to the basic rules of radiation safety provides adequate protection.
The human blood components included in this kit have been tested by European approved and/or FDA approved methods and found negative for HBsAg, anti-HCV, anti-HIV-1 and 2. No known method can offer complete assurance that human blood derivatives will not transmit hepatitis, AIDS or other infections. Therefore, handling of reagents, serum or plasma specimens should be in accordance with local safety procedures.
All animal products and derivatives have been collected from healthy animals. Bovine components originate from countries where BSE has not been reported. Nevertheless, components containing animal substances should be treated as potentially infectious.
Avoid any skin contact with reagents (sodium azide as preservative). Azide in this kit may react with lead and copper in the plumbing and in this way form highly explosive metal azides. During the washing step, flush the drain with a large amount of water to prevent azide build-up.
Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.

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XIX. SUMMARY OF THE PROTOCOL

	TOTAL COUNTS µl	CALIBRATORS µl	SAMPLE(S) CONTROLS µl
Calibrators (0-6) Samples, controls Incubation Buffer	- -	200 - 50	- 200 50
Incubation	2 hour at room temperature with shaking at 400 rpm		
Separation Washing solution Separation Washing solution Separation	- - - - -	aspirate 2.0 aspirate 2.0 aspirate	
Tracer	100	100	100
Incubation	1 hour at room temperature with shaking at 400 rpm		
Separation Washing solution Separation Washing solution Separation	- - - - -	aspirate 2.0 aspirate 2.0 aspirate	
Counting	Count tubes for 60 seconds		

DIAsource Catalogue Nr : KIR0061	P.I. Number : 1700610/RUO	Date of issue : 191227/1

Revision date: 2019-12-27

Distributed by :

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