

# ACTH

Immunoradiometric (IRMA) Assay

(Coated Tube-Technology)

For the quantitative determination  
of human Adrenocorticotrophic hormone

For *in vitro* diagnostic use only



## Scantibodies

(Part Number: 3KG011)

Store at 2 – 8° C

### INTENDED USE

The Scantibodies ACTH IRMA kit has been designed for the quantitative determination of human adrenocorticotrophic hormone levels in human plasma.

### SUMMARY

Adrenocorticotrophic hormone (ACTH) is a single chain polypeptide hormone consisting of 39 amino acids with a molecular weight of 4540 Daltons. The main biological function of ACTH is to increase the synthesis and release of all adrenal steroid hormones. Determination of the plasma ACTH level is useful in clinical investigation of hyper- or hypo- secretion of ACTH associated with the different diseases, such as Addison's disease, Cushing's syndrome, Nelson's syndrome, and adrenal dysfunction.

ACTH originates from a large precursor molecule, *proopiomelanocortin*. Proopiomelanocortin is hydrolyzed to pro-ACTH and  $\beta$ -lipotropin in the anterior lobe of the pituitary gland. Pro-ACTH is further processed to ACTH which is secreted intermittently from the anterior pituitary with nyctohemeral rhythm. Highest levels prevail between 0600 and 0800 hours and lowest levels between 2100 and 2200 hours. Pregnancy, phase of menstrual cycle and stress increase secretion. Significant oscillations throughout the day may also be observed. Circulating ACTH is immunoheterogeneous and has a very short half-life of 5 to 10 minutes. Research has shown that there is ACTH-like immunoreactive peptide in human plasma. It is the "big" form of circulating ACTH, big-ACTH. Big-ACTH is derived not only from a normal human pituitary gland but also from ectopic ACTH producing source. Therefore, samples which contain a high level of big-ACTH in the total ACTH-like immunoreactivity should be carefully evaluated.

Methods for measuring ACTH include bioassay, receptor assay, competitive immunoassay and two-site immunometric assay. Bioassay and receptor assay have limited applications in the clinical laboratory because of the complexity of the procedure and the expense of the reagents. Competitive ACTH immunoassays usually have a poor sensitivity and specificity. Although various techniques of sample extraction can be used to increase assay sensitivity, these procedures often make the ACTH assay more complex, less precise, and more time-consuming than other techniques. Immunometric assays for ACTH have been developed and demonstrate that they are more sensitive, specific, reliable and convenient for use in the

clinical laboratory.

### PRINCIPLE OF PROCEDURE

Scantibodies ACTH kit is an immunoradiometric (IRMA) assay utilizing polyclonal antibodies directed against ACTH 1-16 and ACTH 24-39. The use of these antibodies guarantees that only ACTH is detected. The anti-ACTH 24-39 (C-terminal fragment) is labeled with  $^{125}\text{I}$ . The antibody directed against ACTH 1-16 (N-terminal fragment) is coated onto the tubes. ACTH in patient samples is bound both to the tubes and to the  $^{125}\text{I}$ -anti ACTH (24-39) antibody. After incubation, free  $^{125}\text{I}$ -anti ACTH (24-39) and bound  $^{125}\text{I}$ -anti ACTH (24-39) antibody fractions are separated by discarding the supernatant. Simple wash steps reduce the non-specific binding (NSB) to a minimum for increased precision at the low end of the calibration curve. The concentration of ACTH is directly proportional to the radioactivity bound to the tubes after separation. The concentration of ACTH in unknown patient samples and controls is determined by interpolation using a calibration curve.

### REAGENTS

The Scantibodies ACTH kit contains sufficient reagents for 100 single determinations. The kit is stable at 2 – 8° C until the stated expiration date.

#### ACTH STANDARDS

One set of standards consists of seven vials containing lyophilized serum with synthetic ACTH peptide. The lyophilized standards are prepared in stabilized serum containing sodium azide 0.1% (w/v). The ACTH concentrations are declared on the vial label.

#### ACTH CONTROLS

One set of controls consists of two vials containing ACTH in lyophilized serum with 0.1% (w/v) sodium azide. The concentration ranges of ACTH are declared on the vial labels.

#### ACTH $^{125}\text{I}$ -ANTIBODY

One set of tracer consists of two bottles of  $^{125}\text{I}$ -anti ACTH (24-39). Each bottle contains goat anti-ACTH (24-39) antibody which is labeled with  $^{125}\text{I}$  and dissolved in 5 ml phosphate buffered saline with sodium azide 0.1% (w/v) and protein stabilizers. The maximum radioactivity in a bottle is <370 kBq (<10

μCi).

### **ACTH ANTIBODY COATED TUBES**

Two packages contain 100 polystyrene tubes (12 x 75 mm diameter) plus desiccant. The tubes are coated with goat anti-ACTH (1-16) antibody. The desiccant contains silica.

### **WASH CONCENTRATE**

One bottle contains 30 ml of a 30 fold concentrate of phosphate buffered saline with sodium azide 1.5% (w/v) and detergent.

## **PREPARATION AND STORAGE OF REAGENTS**

### **ACTH STANDARDS**

Reconstitute the zero calibrator with 4 ml of distilled or deionized water. Reconstitute the remaining calibrators with 2 ml of distilled or deionized water. After addition of the water, let the vials sit for 10 minutes at room temperature, mix each vial thoroughly but gently. Use the reconstituted calibrators within 1 hour. Store the unused portion of the calibrators below -20° C until the stated expiration date. Do not store the calibrators at room temperature for more than one hour at any given time. Do not thaw any calibrator vial more than three times.

### **ACTH CONTROLS**

Reconstitute the vials of controls with 2 ml of distilled or deionized water. After addition of the water, let the vials sit for 10 minutes at room temperature, mix each vial thoroughly but gently. Use the reconstituted controls within 1 hour. Store the unused portion of the controls below -20° C until the stated expiration date. Do not store the controls at room temperature for more than one hour at any given time. Do not thaw any control vial more than three times.

### **ACTH <sup>125</sup>I-ANTIBODY**

The tracer is ready to use. Store the tracer at 2 – 8° C until the stated expiration date.

### **ACTH ANTIBODY COATED TUBES**

The antibody coated tubes are ready to use. Store the tubes at 2 – 8° C until the stated expiration date. Allow the tubes to equilibrate to ambient temperature prior to opening the package. Reseal the package immediately after removing the required number of tubes.

### **WASH CONCENTRATE**

Mix the contents of the wash concentrate thoroughly with 870 ml of distilled or deionized water (1:30). Store the diluted wash solution at room temperature (18 - 25° C) until the stated expiration date.

## **WARNINGS AND PRECAUTIONS FOR USERS**

### **Use of The Assay**

The reagents are for in vitro diagnostic use only.

## **Human Serum Caution**

The human serum in this kit has been prepared from human donors and it has been tested by FDA approved immunoassays and found to be non-reactive for Hepatitis B Surface Antigen (HBsAg), Anti HIV I/II and Anti HCV. However, it is recommended to consider the calibrators and controls as a potential biohazard and handle them with the same precautions as applied to any untested patient sample.

## **Radioactivity Warning**

This radioactive material may be received, acquired, possessed, or used only by physicians, clinical laboratories, or hospitals and only for *in vitro* clinical or laboratory tests not involving internal or external administration of the material, or the radiation therefrom, to human beings or animals. Its receipt, acquisition, possession, use and transfer are subject to the regulations and a general license of the U.S. Nuclear Regulatory Commission or of a state with which the Commission has entered into an agreement for the exercise of regulatory authority.

All radioactive materials must be disposed of according to the regulations (regulations differ from country to country) and guidelines of the agencies with jurisdiction over the laboratory. Do not eat, drink, smoke or apply cosmetics in areas where radioactive materials are used. Storage of radioactive materials should be limited to specifically designated and appropriately secured areas. Access to radioactive materials should be limited to authorized and trained personnel only. Do not pipette radioactive solutions by mouth. Avoid direct contact with radioactive materials by using protective articles such as lab coats and disposable gloves. Radioactive materials must be stored in designated areas in their original containers or in containers providing equivalent radiation protection. A record of disposal of all radioactive materials must be kept. Immediately remove spilled solutions and decontaminate contaminated devices. Check laboratory equipment and glassware regularly to detect contamination with radioisotopes.

## **Sodium Azide (NaN<sub>3</sub>) Warning**

Some reagents in the Scantibodies ACTH assay contain sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal flush the drain with a large volume of water to prevent azide build-up. Avoid direct contact with skin and mucous membranes.

## **SAMPLE PREPARATION AND STORAGE**

### **Specimen Collection**

The determination of human ACTH must be made on EDTA-plasma. Do not use serum or heparinized plasma sample. Four hundred microliters of EDTA-plasma are required to assay one sample in duplicate. To obtain EDTA-plasma, collect blood by venipuncture in either siliconized glass tubes with EDTA as the anticoagulant (lavender top Vacutainer, Becton Dickinson Cat.# 6456, or

equivalent) or in a plastic syringe where the blood can be transferred to a siliconized tube containing EDTA (7.5 mg/5mL whole blood). Centrifuge the sample and separate the plasma from the cells with a refrigerated centrifuge as soon as possible. EDTA-plasma should be stored at -20° C or colder immediately after separation from cells. Avoid any repeated freezing and thawing cycles.

For meaningful evaluation or comparisons, blood samples should be drawn at the same time each day.

#### Dilution of Patient Samples

Dilute EDTA-plasma samples with ACTH concentrations greater than the highest calibrator with Scantibodies ACTH Zero Calibrator before assay. The dilution factor is applied to the diluted sample assay result in order to determine the ACTH concentration in the undiluted sample.

#### QUALITY CONTROL

Two levels of controls are provided with each assay kit. The values assigned to these controls are printed on the container label. The control value should fall within the specified range when tested in the same manner as the unknowns. Control should be included in each assay. If the control values do not meet the established range, the assay may be invalid and should be repeated.

#### ASSAY PROCEDURE

##### Materials Provided

The Scantibodies ACTH kit (Part No. 3KG011) is supplied with the following:

DESCRIPTION	NUMBER
ACTH Standards Part Nos. 3CA460, 3CB460, 3CC460, 3CD460, 3CE460, 3CF460, 3CG460	7 vials
ACTH Controls Part Nos. 3CA461, 3CB461	2 vials
ACTH Antibody Coated Tubes Part No. 3KT050	2 packages of 50 tubes each
ACTH <sup>125</sup> I-Antibody Part No. 3KL125	2 vials
Wash Concentrate Part No. 3KW001	1 bottle
Directional Insert Part No. 7KI015	1 insert

##### Materials and Equipment Required But Not Provided:

Distilled or deionized water  
Round-bottomed polypropylene or polystyrene test tubes (12 x 55, 12 x 75, 12 x 70 mm or equivalent)  
Pipettor with disposable tips: 0.2 ml  
Wash station  
Tube shaker capable of 170 RPM  
Vortex mixer  
Gamma counter calibrated to detect <sup>125</sup>I

##### Preparation for Assay

For each assay, prepare the following groups of tubes and place them in a test tube rack (double determination):

- 2 total count tubes (optional for QC). Use non-coated tubes.
- 2 Bo tubes (NSB)
- 2 tubes for each calibrator concentration
- 2 tubes for each control concentration
- 2 tubes for each patient sample

#### Pipetting and Incubation Steps

- Pipette 0.2 ml of calibrators, samples and controls into the corresponding tubes.
- Pipette 0.1 ml of Scantibodies ACTH <sup>125</sup>I-antibody into each tube.
- Gently vortex all tubes.
- Seal the tubes and incubate them for 18 - 22 hours at room temperature (18 - 25° C) with shaking at 170 RPM.
- Aspirate the supernatant from each tube except for the total count tubes. Wash the tubes 3 times with 2 ml of diluted wash solution. After each addition of diluted wash solution aspirate all of the wash solution.
- Count each tube for at least 1 minute in a gamma counter calibrated to detect <sup>125</sup>I. The total count tube should contain approximately 300,000 CPM (assuming the counter has an efficiency of 70% - 80%) when freshly iodinated ACTH <sup>125</sup>I-antibody is used. The total activity of the tracer decreases according to the half-life of <sup>125</sup>I.

#### PIPETTING GUIDE

Additive to Tube	Total Count Tubes	Bo Tubes	Calibrator Tubes	Control Tubes	Sample Tubes
Standard	-	200 µl	200 µl		-
Control	-	-	-	200 µl	
Sample	-	-	-	-	200 µl
ACTH <sup>125</sup> I-Antibody	100 µl	100 µl	100 µl	100 µl	100 µl
Vortex mix all tubes, except for the total count tubes. Incubate tubes for 18 - 22 hours at room temperature (18 - 25° C) and shaking 170 RPM.					
Aspirate the supernatant from all of the tubes except the total count tubes. Wash all tubes except the total count tubes by adding 2 ml of diluted wash solution and aspirating the wash solution. Repeat this wash step two more times for a total of three times.					
Count each tube for at least 1 minute in a gamma counter.					

#### PROCEDURAL COMMENTS

##### Known Interferences:

Samples containing up to 250 mg/dl triglyceride, 15 mg/dl hemoglobin and 15 mg/dl Bilirubin do not exhibit any effect

on the assay.

Grossly hemolyzed or lipemic samples.

Samples from patients receiving radioisotopes for diagnostic or therapeutic purposes.

Contamination of the sample or assay tube with  $^{125}\text{I}$  or other radioisotopes.

**Reagents from different lot numbers must not be interchanged.**

The patient sample or calibrator and the ACTH  $^{125}\text{I}$ -antibody should be pipetted carefully into the bottom one-fourth of the assay tube. This is to avoid losing liquid on the surface of the tube as the liquid runs down the tube.

The washing step is an important step in the assay procedure. Accurate dispensing of the wash solution and complete aspiration of the tube contents is essential to achieving assay sensitivity, low background and assay precision.

It is recommended that calibrators and patient samples be assayed in duplicate. The average counts per minute of each duplicate should then be used for data reduction and the calculation of results.

Avoid sample to sample contamination by using a new pipette tip for each sample.

## CALCULATION OF RESULTS

### Evaluation

1. Calculate the average CPM for each double determination.
2. Subtract the average CPM of the zero calibrator tubes from the CPM's from all other tubes in order to obtain the corrected CPM for each tube.
3. Corrected CPM = average CPM of duplicate samples - average CPM of duplicate zero calibrators.
4. Draw the calibration curve by plotting the average corrected CPM from each duplicate calibrator level (ordinate) against the respective concentration declared on the calibrator vial (absolute) using log-log graph paper. Obtain sample concentrations by interpolation of average sample CPM on the calibration curve.
5. If samples were run with dilution, multiply the diluted sample assay results from the curve by the appropriate dilution factors to obtain the undiluted sample assay results.

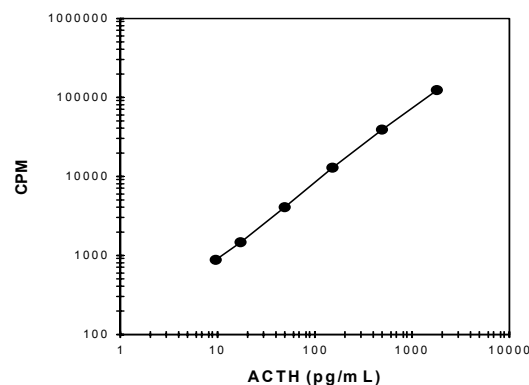
### SAMPLE DATA

Tube	CPM	Ave. CPM	Corrected CPM
Total Activity	287160 289659	288409	
0 pg/ml	224 206	215	
9.5 pg/ml	807	804	589

	802		
17 pg/ml	1335 1422	1379	1164
50 pg/ml	3586 3755	3670	3455
150 pg/ml	10864 10866	10865	10650
500 pg/ml	34001 34711	34356	34141
1800 pg/ml	106754 104721	105737	105522

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

### REPRESENTATIVE STANDARD CURVE



Automated data reduction can also be used to construct the Scantibodies ACTH calibration curve. To program automated data reduction systems or to adapt an existing program consult with the data processor manufacturer or the programmer.

### LIMITATIONS OF THE PROCEDURE

For diagnostic purposes ACTH values should be used in addition to other diagnostic data and clinical information available to the physician.

The assay procedure must be followed exactly; careful technique must be used to obtain valid results. Any modification of the assay procedure is likely to alter the results.

Grossly hemolyzed, lipemic or icteric samples are likely to give non valid results.

The highest concentration of ACTH measurable without sample dilution is the concentration of the highest calibrator. The lowest level measurable is approximately 1.0 pg/ml.

### EXPECTED VALUES

It is recommended that each laboratory establish its own range of normal values. The values given are only

indicative and may vary from other published data.

EDTA-plasma from 120 apparently healthy, fasting adults were drawn between the hours of 8 a.m. to 12 p.m. Plasma ACTH levels were measured with said ACTH assay. Consistent with NCCLS guideline (C28-A), the normal range of ACTH in this normal group was between 5 to 77 pg/ml. The central 95% reference interval calculation was utilized to obtain the normal range.

## PERFORMANCE CHARACTERISTICS

### Accuracy, Recovery

Different samples with concentrations of ACTH were spiked with 3 amounts of ACTH. The % recovery was determined following assay of the spiked samples.

ACTH IRMA					
Accuracy, Recovery					
Sample	Sample value (pg/ml)	Added ACTH (pg/ml)	Measured value (pg/ml)	Expected value (pg/ml)	Recovery (%)
1	35.81	40.0	95.77	75.81	126
		80.0	142.27	115.81	123
		120.0	182.98	155.81	117
2	670.16	40.0	719.58	710.16	101
		80.0	756.57	750.16	101
		120.0	784.27	790.16	99
3	1434.1	40.0	1470.3	1474.1	99.7
		80.0	1469.4	1514.1	97
		120.0	1494.6	1554.1	96

### Accuracy, Dilution

Different samples with high concentrations of ACTH were diluted in a sera with zero calibrator. The % recovery was determined following assay of the diluted samples.

ACTH				
Accuracy, Dilution				
Sample	Dilution	Measured value (pg/ml)	Expected value (pg/ml)	Recovery (%)
1	Neat	336.60	-	-
	1 to 2	170.20	168.3	101.1
	1 to 4	91.67	84.15	108.9
	1 to 8	45.43	42.08	108.0
2	Neat	1050.40	-	-
	1 to 2	471.75	525.2	89.8
	1 to 4	234.30	262.6	89.2
	1 to 8	131.71	131.3	100.3
3	Neat	1327.10	-	-
	1 to 2	658.17	663.55	99.2
	1 to 4	333.98	331.78	100.7
	1 to 8	180.99	165.89	109.1
	1 to 16	101.13	82.94	121.9

## Precision

Intra-assay coefficient of variation was evaluated by performing 20 replicate determinations on 3 EDTA-plasma pools in the same assay.

ACTH				
Precision, Intra-assay				
Kit Batch	Sample	Mean value (pg/ml)	Std. Dev. (pg/ml)	% CV
E1	1	30.76	2.39	7.76
E1	2	616.39	5.61	0.91
E1	3	1417.72	27.38	1.93
E2	1	37.38	2.99	8.00
E2	2	671.50	14.44	2.15
E2	3	1484.68	29.22	1.97
E3	1	29.11	2.33	8.00
E3	2	614.87	21.83	3.55
E3	3	1425.17	30.74	2.16

Inter-assay coefficient of variation was evaluated by performing 20 different assays on 4 EDTA-plasma pools.

ACTH				
Precision, Inter-assay				
Kit Batch	Sample	Mean value (pg/ml)	Std. Dev. (pg/ml)	% CV
E1	1	31.11	3.67	11.78
E1	2	234.84	15.59	6.64
E1	3	643.17	27.24	4.24
E1	4	1425.39	38.52	2.70
E2	1	31.14	4.77	15.31
E2	2	232.89	9.84	4.23
E2	3	641.81	33.08	5.15
E2	4	1436.86	48.79	3.40
E3	1	31.33	3.94	12.59
E3	2	230.68	10.93	4.74
E3	3	641.64	34.33	5.35
E3	4	1438.42	54.27	3.77

## Sensitivity

The detection limit of the assay is defined as the lowest measurable value distinguishable from zero. This sensitivity was determined by assaying the zero calibrator 20 times in the same assay. The detection limit is approximately  $\leq 1.0$  pg/ml. The 1.0 pg/ml value was used as a conservative estimate of sensitivity where data generated supported a sensitivity of a lower value.

## Specificity

The specificity of Scantibodies ACTH assay was determined from testing the cross-reactivity with other synthetic peptides. Assay standard zero was spiked with

those peptides and measured.

<b>ACTH</b>		
Specificity		
Peptides	Dose Added (pg/mL)	Measurement ACTH Assay (pg/mL)
ACTH (18-39)	100,000	0
	10,000	0
	1,000	0
	500	0
	250	0
ACTH (1-10)	100,000	0
	10,000	0
	1,000	0
	500	0
	250	0
ACTH (1-24)	100,000	0
	10,000	0
	1,000	0
	500	0
	250	0
$\alpha$ -MSH	100,000	0
	10,000	0
	1,000	0
	500	0
	250	0
$\beta$ -MSH	100,000	0
	10,000	0
	1,000	0
	500	0
	250	0
$\beta$ -Endorphin	100,000	0
	10,000	0
	1,000	0
	500	0
	250	0

### High Dose Hook Response

The high dose hook response of the ACTH kit was determined as 25,000 pg/ml of ACTH. Samples greater than the highest standard (approximately 1800 pg/ml) and up to 25,000 pg/ml will read CPM values greater than that of the highest standard.

### Correlation

A high degree of correlation exists between the ACTH levels of duplicate samples measured by a commercially available predicate ACTH kit and those levels measured by the Scantibodies Laboratory, Inc. (SLI) ACTH Specific IRMA Assay. A correlation coefficient (r) of 0.98 (n=161) was obtained with a slope of 1.06 and intercept of 3.76 where x represents the predicate device data and y represents the SLI data. Calculations were made with samples ranging from 4 - 2022 pg/ml.

<b>Chemical Characterization:</b>	1) Antibodies coated on to polystyrene Beads (or tubes).
	2) Radioactive Isotope containing Iodine-125 with radioactivity <10 $\mu$ Ci and Sodium Azide @ 0.1%.
	3) Calibrators & Controls – Human Serum containing Sodium Azide @ 0.1%
	4) Wash Concentrate containing sodium azide @ 1.5%.
<b>Hazardous Ingredients:</b>	Radioactive Isotope (Iodine-125) @ <10 $\mu$ Ci/Vial (<370 kBq) CAS Number: 7553-56-2 Symbols: Harmful Xn R-phrases: R22, R52/53 S-phrases: S28, S45, S53, S60, S61
	Sodium Azide @ 0.1% CAS Number: 026628-22-8 Symbols: Harmful Xn R-phrases: R22, R31, R52/53 S-phrases: S28, S45, S53, S60, S61
	Sodium Azide @ 1.5% CAS Number: 026628-22-8 Symbols: Very Toxic T+; N R-phrases: R28, R32, R50/53 S-phrases: S28, S45, S53, S60, S61

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