Snibe Diagnostic

CE REF 130298003M: 100 tests
130698003M: 50 tests

MAGLUMI® ACTH (CLIA)

INTENDED USE

The kit is an *in vitro* chemiluminescence immunoassay for the quantitative determination of Adrenocorticotropic Hormone (ACTH) in human plasma with the MAGLUMI series Fully-auto chemiluminescence immunoassay analyzer (including Maglumi 600, Maglumi 800, Maglumi 1000, Maglumi 1000 Plus, Maglumi 2000, Maglumi 2000 Plus, Maglumi 4000, Maglumi 4000 Plus, MAGLUMI X8, MAGLUMI X3 and MAGLUMI X6) and Biolumi series Integrated System (including Biolumi CX8).

SUMMARY AND EXPLANATION OF THE TEST

Adrenocorticotropic hormone (ACTH), also known as corticotropin is a polypeptide tropic hormone produced and secreted by the anterior pituitary gland[1]. It is also used as a medication and diagnostic agent. It is an important component of the hypothalamic-pituitary-adrenal axis and is often produced in response to biological stress (along with its precursor corticotropin-releasing hormone from the hypothalamus). Its principal effects are increased production and release of cortisol by the cortex of the adrenal gland. ACTH is also related to the circadian rhythm in many organisms[2].
ACTH stimulates secretion of glucocorticoid steroid hormones from adrenal cortex cells, especially in the zona fasciculata of the adrenal glands. ACTH acts by binding to cell surface ACTH receptors, which are located primarily on adrenocortical cells of the adrenal cortex. The ACTH receptor is a seven-membrane-spanning G protein-coupled receptor[3]. Upon ligand binding, the receptor undergoes conformation changes that stimulate the enzyme adenylyl cyclase, which leads to an increase in intracellular cAMP and subsequent activation of protein kinase A[4].
Deficiency of ACTH is a sign of secondary adrenal insufficiency (suppressed production of ACTH due to an impairment of the pituitary gland or hypothalamus, cf. hypopituitarism) or tertiary adrenal insufficiency (disease of the hypothalamus, with a decrease in the release of corticotropin releasing hormone CRH)[5]. Conversely, chronically elevated ACTH levels occur in primary adrenal insufficiency (e.g. Addison's disease) when adrenal gland production of cortisol is chronically deficient. In Cushing's disease a pituitary tumor is the cause of elevated ACTH (from the anterior pituitary) and an excess of cortisol (hypercortisolism) – this constellation of signs and symptoms is known as Cushing's syndrome[6-7].

PRINCIPLE OF THE TEST

The ACTH assay is a sandwich chemiluminescence immunoassay.
The sample (or calibrator/control, if applicable), ABEI labeled with anti-ACTH monoclonal antibody, magnetic microbeads coated with another anti-ACTH monoclonal antibody are mixed thoroughly and incubated, forming sandwich complexes. After precipitation in a magnetic field, decant the supernatant, and then perform a wash cycle. Subsequently, the Starter 1+2 are added to initiate a chemiluminescent reaction. The light signal is measured by a photomultiplier as relative light units (RLUs), which is proportional to the concentration of ACTH present in the sample (or calibrator/control, if applicable).

KIT COMPONENTS

Material Provided

Components	Contents	100 tests (REF: 130298003M)	50 tests (REF: 130698003M)
Magnetic Microbeads	Coated with anti- ACTH monoclonal antibody, containing BSA, NaN_3 (<0.1%).	2.5 mL	2.0 mL
Calibrator Low	ACTH antigen, containing BSA, NaN_3 (<0.1%).	3.0 mL	2.0 mL
Calibrator High	ACTH antigen, containing BSA, NaN_3 (<0.1%).	3.0 mL	2.0 mL
ABEI Label	Anti-ACTH monoclonal antibody labeled ABEI, containing BSA, NaN_3 (<0.1%).	22.5 mL	12.5 mL
Internal Quality Control	ACTH antigen, containing BSA, NaN_3 (<0.1%).	2.0 mL	2.0 mL
All reagents are provided ready-to-use.			

Accessories Required But Not Provided

MAGLUMI and Biolumi Series:

Reaction Module	REF: 630003
Starter 1+2	REF: 130299004M, 130299027M
Wash Concentrate	REF: 130299005M
Light Check	REF: 130299006M
Reaction Cup	REF: 130105000101

Please order accessories from Shenzhen New Industries Biomedical Engineering Co., Ltd. (SNIBE) or our authorized representatives.

CALIBRATION

Traceability: This method has been standardized against SNIBE internal reference material.
Test of assay specific calibrators allows the RLU values to adjust the assigned master curve. Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve (10 calibrations) provided via the reagent Radio Frequency Identification (RFID) CHIP.
Recalibration is recommended if any of the following conditions occurs:

- After each change of lots (Reagent or Starter 1+2).
- Every week and/or each time a new reagent kit is used (recommended).
- After instrument service is required.
- If control results lie outside the expected range.

QUALITY CONTROL

Follow government regulations or accreditation requirements for quality control frequency.
Internal quality control is only applicable with MAGLUMI and Biolumi systems. For instructions for use and target value refer to ***ACTH (CLIA) Quality Control Information***. User needs to judge results with their own standards and knowledge.
For detailed information about entering quality control values, refer to the corresponding Analyzer Operating Instructions.
To monitor system performance and chart trends, commercially available quality control materials are required. Treat all quality control samples the same as patient samples. A satisfactory level of performance is achieved when analyte values obtained are within the acceptable Control Range for the system or within your range, as determined by an appropriate internal laboratory quality control scheme. If the quality control results do not fall within the Expected Values or within the laboratory's established values, do not report results. Take the following actions:

- Verify that the materials are not expired.
- Verify that required maintenance was performed.
- Verify that the assay was performed according to the instructions for use.
- Rerun the assay with fresh quality control samples.
- If necessary, contact your local technical supporters or distributors for assistance.

SPECIMEN COLLECTION AND PREPARATION

- Sample material: plasma.
- Collect blood with anticoagulation blood tube (EDTA-K2), then put the tube into ice-bath, use low-temperature centrifuge to separate the plasma from the rest and put the plasma into -20°C for storage.
- Inspect all specimens for bubbles, and remove bubbles before analysis for optimal results. Centrifuged specimens with a lipid layer on the top must be transferred to a sample cup or a secondary tube. Care should be taken to transfer only the clarified specimen without the lipemic material.
- All samples (patient specimens and controls) should be tested within 3 hours when placed on board the MAGLUMI and Biolumi Systems. Refer to the SNIBE service for more details of onboard sample storage constraints.
- If testing will be delayed for more than 3 hours, remove plasma from the plasma separator, red blood cells or clot. Plasma specimen removed from the separator, cells or clot was stable at room temperature for 20 hours; store at 2-8°C for 20 hours; for longer storage periods (4 weeks), freeze to below -20°C.
- Before shipping specimens, it is recommended that specimens be removed from the plasma separator, red blood cells or clot. When shipped, specimens should be packaged and labeled in compliance with applicable state, federal and international regulations covering the transport of clinical specimens and infectious substances. Specimens should be shipped frozen.
- The sample volume required for a single determination of ACTH is 200 µL.

WARNING AND PRECAUTIONS FOR USERS

- IVD
- For *In Vitro* Diagnostic Use.
- Follow the package insert carefully. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Safety Precautions

- **CAUTION:** This product requires the handling of human specimens. It is recommended that all human sourced materials be considered potentially infectious and handled in accordance with the 29 CFR 1910.1030 Occupational exposure to bloodborne pathogens. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.
- All samples, biological reagents and materials used in the assay should be considered potentially able to transmit infectious agents. They should therefore be disposed in accordance with the practices of your institution. Discard all materials in a safe and acceptable manner and in compliance with prevailing regulatory requirements.
- This product contains Sodium Azide. Dispose of contents and containers must be in accordance with all local, regional and national regulations.
- Refer to safety data sheets which are available on request.

Handling Precautions

- Do not use reagent kits beyond the expiration date.
- Do not interchange reagent components from different reagents or lots.
- Prior to loading the Reagent Kit on the system for the first time, the Reagent Kit requires mixing to re-suspend magnetic microbeads that have settled during shipment.
- For magnetic microbeads mixing instructions, refer to the Preparation of the Reagent section of this package insert.
- To avoid contamination, wear clean gloves when operating with a reagent kit and samples.
- Over time, residual liquids may dry on the septum surface. These are typically dried salts which have no effect on assay efficacy.
- For detailed discussion of handling precautions during system operation, refer to the SNIBE service information.

STORAGE AND STABILITY

- Sealed: Stored at 2-8°C until the expiration date.
- Opened at 2-8°C: Minimum stability is 4 weeks.
- On-board: Minimum stability is 4 weeks.
- To ensure the best kit performance, it is recommended to place opened kits in the refrigerator after the end of the intraday test work.
- Keep upright for storage to facilitate later proper resuspension of magnetic microbeads.
- Keep away from sunlight.

TEST PROCEDURE

Preparation of the Reagent

- Resuspension of the magnetic microbeads takes place automatically when the kit is loaded successfully, ensuring the magnetic microbeads are totally resuspended homogenous prior to use.
- To ensure proper test performance, strictly adhere to the corresponding Analyzer Operating Instructions. Each test parameter is identified via a RFID CHIP on the Reagent kit. For further information please refer to the corresponding Analyzer Operating Instructions.

DILUTION

Sample dilution by analyzer is not available in this reagent kit.
Samples with concentrations above the measuring range can be diluted manually. After manual dilution, multiply the result by the dilution factor. Please choose applicable diluents or ask SNIBE for advice before manual dilution.

High-Dose Hook

No high-dose hook effect was seen for ACTH concentrations up to 50,000 pg/mL.

LIMITATIONS

- A skillful technique and strict adherence to the instructions are necessary to obtain reliable results.
- Bacterial contamination or heat inactivation of the specimens may affect the test results.
- A result within the expected range does not rule out the presence of disease and should be interpreted together with other diagnostic procedures.
- Test results are reported quantitatively. However, diagnosis of a disease should not be based on the result of a single test, but should be determined in conjunction with clinical findings in association with medical judgement.
- Any therapeutical decision should also be taken on a case-by-case basis.
- Patient samples containing human anti-mouse antibodies (HAMA) may give falsely elevated or decreased values. Although HAMA-neutralizing agents are added, extremely high HAMA plasma concentrations may occasionally influence results.
- Patients with malignancies may exhibit ACTH values within the normal range. ACTH concentrations may be elevated in case of liver cirrhosis, hepatitis or tyrosinemia. Thus, ACTH determination is more suitable for therapeutic monitoring and follow-up as well as for a comparison with histological results. ACTH levels may only be interpreted in context with the clinical symptoms and other diagnostic procedures.

RESULTS

Calculation of Results

The analyzer automatically calculates the ACTH concentration of each sample by means of a calibration curve which is generated by a 2-point calibration master curve procedure. The results are reported in the unit of pg/mL. For further information please refer to the corresponding Analyzer Operating Instructions.

Interpretation of Results

The expected ranges for the ACTH assay were obtained by testing 357 apparent healthy individuals at different time gave the following expected values:

Time	N	2.5^{th}-97.5^{th} percentiles (pg/mL)
8:00-10:00	115	6-48
16:00	126	3-30

Time	N	95^{th} percentile (pg/mL)
24:00	116	<20

Results may differ between laboratories due to variations in population and test method. It is recommended that each laboratory should establish its own expected ranges.

PERFORMANCE CHARACTERISTICS

Precision

Precision for the ACTH assay was determined as described in the CLSI EP5-A2. 3 human plasma pools and 3 controls containing different concentration of analyte were assayed in duplicate at two independent runs per day for 20 testing days. The results are summarized in the following table:

Sample	Mean(pg/mL) (N=80)	Within-Run		Between-Run		Total	
		SD(pg/mL)	%CV	SD(pg/mL)	%CV	SD(pg/mL)	%CV
Plasma Pool 1	6.000	0.290	4.83	0.258	4.30	0.388	6.47
Plasma Pool 2	50.190	1.966	3.92	1.620	3.23	2.548	5.08
Plasma Pool 3	702.558	16.953	2.41	16.729	2.38	23.817	3.39
Control 1	39.939	1.913	4.79	1.238	3.10	2.279	5.71
Control 2	200.807	6.325	3.15	6.226	3.10	8.876	4.42
Control 3	601.173	17.108	2.85	17.092	2.84	24.184	4.02

Limit of Blank (LoB)

The LoB for the ACTH assay is 0.5 pg/mL.

Limit of Detection (LoD)

The LoD for the ACTH assay is 1.5 pg/mL.

Measuring Range

0.5-2000 pg/mL (defined by the limit of blank and the maximum of the master curve). Values below the limit of blank are reported as <0.5 pg/mL. Values above the measuring range are reported as >2000 pg/mL.

Linearity

The assay is linear between 1.5 pg/mL and 2000 pg/mL based on a study performed with guidance from CLSI EP6-A. Nine equally distributed levels of samples were prepared by blending a plasma sample containing ACTH 2100 pg/mL with a plasma sample depleted of ACTH (0.0 pg/mL).The mean sample recovery ranged between 90% to 110%.

Method Comparison

A total of 128 samples in the range of 1.968 and 1934.03 pg/mL were tested using the ACTH assay (y) and a commercially available immunoassay (x). The data from the resulting linear regressions are summarized as:y=1.063x-6.3269. r^2=0.9884.

Analytical Specificity

The specificity of the assay was obtained by adding PTH (200 pg/mL) to two plasma samples at the indicated concentrations. No interference was found.

Endogenous Interference
Substances up to the following concentrations did not interfere with the assay:

- Bilirubin 25 mg/dL
- Hemoglobin 400 mg/dL
- Triglyceride 1500 mg/dL

REFERENCES

1. I.K. Morton; Judith M. Hall (December 6, 2012). Concise Dictionary of Pharmacological Agents: Properties and Synonyms. Springer Science & Business Media. pp. 84–.
2. Dibner C, Schibler U, Albrecht U (2010). "The mammalian circadian timing system: organization and coordination of central and peripheral clocks". Annual Review of Physiology. 72: 517–49.
3. Raikhinstein M, Zohar M, Hanukoglu I (February 1994). "cDNA cloning and sequence analysis of the bovine adrenocorticotropic hormone (ACTH) receptor". Biochimica et Biophysica Acta. 1220 (3): 329–32.
4. Hanukoglu I, Feuchtwanger R, Hanukoglu A (November 1990). "Mechanism of corticotropin and cAMP induction of mitochondrial cytochrome P450 system enzymes in adrenal cortex cells" (PDF). The Journal of Biological Chemistry. 265 (33): 20602–8.
5. Demitrack, M. A., Dale, J. K., Straus, S. E., Laue, L., Listwak, S. J., Kruesi, M. J., ... & Gold, P. W. (1991). Evidence for impaired activation of the hypothalamic-pituitary-adrenal axis in patients with chronic fatigue syndrome. *The Journal of Clinical Endocrinology & Metabolism*, *73*(6), 1224-1234.
6. Oelkers, W., Diederich, S., & Bähr, V. (1992). Diagnosis and therapy surveillance in Addison's disease: rapid adrenocorticotropin (ACTH) test and measurement of plasma ACTH, renin activity, and aldosterone. *The Journal of Clinical Endocrinology & Metabolism*, *75*(1), 259-264.
7. Arlt, W., & Allolio, B. (2003). Adrenal insufficiency. *The Lancet*, *361*(9372), 1881-1893.

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SYMBOLS EXPLANATIONS

	Consult instructions for use		Manufacturer
2°C 8°C	Temperature limit (Store at 2-8 °C)		Use-by date
Σ	Contains sufficient for		Keep away from sunlight
	This way up	EC REP	Authorized representative in the European Community
IVD	*In vitro* diagnostic medical device	CONTENTS	Kit components
REF	Catalogue number	LOT	Batch code