Snibe Diagnostic

CE

REF 130298002M: 100 tests
130698002M: 50 tests

MAGLUMI® Cortisol (CLIA)

INTENDED USE

The kit is an *in vitro* chemiluminescence immunoassay for the quantitative determination of cortisol in human serum or urine using 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

Cortisol is a steroid hormone, in the glucocorticoid class of hormones. It is produced in humans by the zona fasciculata of the adrenal cortex within the adrenal gland[1]. It is released in response to stress and low blood-glucose concentration. It functions to increase blood sugar through gluconeogenesis, to suppress the immune system, and to aid in the metabolism of fat, protein, and carbohydrates[2-3]. It also decreases bone formation[2].

The release of cortisol is controlled by the hypothalamus, a part of the brain. The secretion of corticotropin-releasing hormone by the hypothalamus triggers cells in the neighboring anterior pituitary to secrete another hormone, the adrenocorticotropic hormone (ACTH), into the vascular system, through which blood carries it to the adrenal cortex. ACTH stimulates the synthesis of cortisol, glucocorticoids, mineralocorticoids, and dehydroepiandrosterone[4-6]. The primary control of cortisol is the pituitary gland peptide, ACTH, which probably controls cortisol by controlling the movement of calcium into the cortisol-secreting target cells[7]. ACTH is in turn controlled by the hypothalamic peptide corticotropin-releasing hormone (CRH), which is under nervous control. CRH acts synergistically with arginine vasopressin, angiotensin II, and epinephrine[8].

Like most steroids cortisol is not very water soluble so is transported around the body bound to a protein, an α2-globulin, known as cortisol-binding globulin (CBG) or transcortin. In blood, ~92% cortisol is bound to CBG and ~8% is the non-protein-bound “free” hormone, which is the biologically active component[4,9]. Cortisol measurements are used as a direct monitor of adrenal status and an indirect measure of pituitary hyper or hypofunction. Elevated serum levels can be found in stress responses, psychiatric diseases, obesity, diabetes, alcoholism and pregnancy, which may cause diagnostic problems in patients with Cushing's syndrome. Low levels of cortisol are seen in patients with generalized adrenal hypofunction or a defect in the metabolic pathway for cortisol biosynthesis[9-11].

PRINCIPLE OF THE TEST

The Cortisol assay is a competitive chemiluminescence immunoassay.

The sample (or calibrator/control, if applicable), ABEI labeled with purified cortisol antigen, FITC labeled with anti-cortisol monoclonal antibody and magnetic microbeads coated with sheep anti-FITC polyclonal antibody are mixed thoroughly and incubated, forming immuno-complexes. After precipitation in a magnetic field, the supernatant is decanted and then a wash cycle is performed. 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 inversely proportional to the concentration of cortisol present in the sample (or calibrator/control, if applicable).

KIT COMPONENTS

Material Provided

Components	Contents	100 tests (REF: 130298002M)	50 tests (REF: 130698002M)
Magnetic Microbeads	Coated with sheep anti-FITC polyclonal antibody, NaN_3 (<0.1%).	2.5 mL	2.0 mL
Calibrator Low	Cortisol antigen, containing BSA, NaN_3 (<0.1%).	2.5 mL	2.0 mL
Calibrator High	Cortisol antigen, containing BSA, NaN_3 (<0.1%).	2.5 mL	2.0 mL
FITC Label	Anti-cortisol monoclonal antibody labeled FITC, containing BSA, NaN_3 (<0.1%).	10.5 mL	7.0 mL
ABEI Label	Cortisol antigen labeled ABEI, containing BSA, NaN_3 (<0.1%).	10.5 mL	7.0 mL
Internal Quality Control	Cortisol 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 the ID-GC/MS (isotope dilution gas chromatography mass spectrometry).

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 two weeks and/or each time a new reagent kit is used (recommended).
- After instrument service is required.

- If controls 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 ***Cortisol (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: serum

- Use standard sampling tubes or tubes containing separating gel. Collect blood aseptically following the universal precautions for venipuncture.
- Ensure that complete clot formation in specimens has taken place prior to centrifugation. Some specimens, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time.
- If the specimen is centrifuged before a complete clotting, the presence of fibrin may cause erroneous results. Samples must be free of fibrin and other particulate matter.
- Do not use hemolyzed or grossly lipemic specimens as well as specimens containing particulate substance or exhibiting obvious microbial contamination. Inspect all specimens for bubbles, and remove bubbles before analysis for optimal results.
- Avoid repeated freezing and thawing. The serum sample can be frozen and thawed for two times. Stored samples should be thoroughly mixed prior to use (Vortex mixer). Frozen specimens must be mixed THOROUGHLY after thawing by LOW speed vortexing. Please ask local representative of SNIBE for more derails if you have any doubt.
- Centrifuged specimens with a lipid layer on the top must be transferred to a sample cup or secondary tube. Care should be taken to transfer only the clarified specimen without the lipaemic material.
- Specimens removed from the separator, red blood cells or clot may be stored up to 7 days at 2-8°C, and stored up to 3 months frozen at -20°C or colder.

Sample material: urine

- Collect fresh urine into a clean container within 24 hours, there should be no antiseptic substance inside it, or add 10g boric acid into the urine sample by each liter.
- Blend and mix the sample completely before measurement, certain sample that contains specific granule should be centrifuged prior to use.
- The urine sample can be frozen and thawed for two times and may be stored up to 24 hours at 2-8°C, and stored up to 3 months frozen at -20°C or colder.
- 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.
- Before shipping specimens, it is recommended that specimens be removed from the clot, red blood cells, or separator. 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. Serum specimens should be shipped frozen.
- The sample volume required for a single determination of cortisol is 40 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. It is still possible to keep on using the kit beyond the opened or on-board period if the controls are found within the expected ranges.
- 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.

LIMITATIONS

- A skillful operation and strict adherence to the instructions are necessary to obtain reliable results. Procedural directions should be followed exactly and careful operation should be used to obtain valid results. Any modification of the procedure is likely to alter the results.
- For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the patient sample. Patients who have been regularly exposed to animals or received immunotherapy may contain human anti-mouse antibodies (HAMA), which may result in falsely elevated or decreased values. Moreover, other heterophile antibodies such as human anti-goat antibodies may be present in patient samples as well. Additional clinical or diagnostic information may be required to determine patient status.
- Bacterial contamination or repeated freeze-thaw cycles may affect the test results.
- Due to the diurnal variation of cortisol levels in normal subjects, all serum cortisol measurements should be referenced to the time of day of sample collection.

RESULTS

Calculation of Results

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

Interpretation of Results

Serum:

The expected ranges for serum cortisol were obtained by testing 125 apparently healthy individuals at different time in China, and gave the following expected values:

Collection Time	N	2.5^{th}-97.5^{th} percentiles (ng/mL)
8:00-10:00	125	57.2-194.2
16:00-18:00	125	20.2-131.0

Urine:

The expected range for urine cortisol was obtained by testing 120 apparently healthy individuals in China, and gave the following expected values: 30-350 μg/24-hour.
μg/24-hour=(Concentration in ng/mL)×(Volume of urine excreted in milliliter per 24 hours)×10^{-3}.
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 Cortisol assay was determined as described in the CLSI EP5-A2. 3 human serum 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(ng/mL) (N=80)	Within-Run		Between-Run		Total	
		SD(ng/mL)	%CV	SD(ng/mL)	%CV	SD(ng/mL)	%CV
Serum Pool 1	51.866	2.682	5.17	3.629	7.00	4.512	8.70
Serum Pool 2	156.278	7.631	4.88	5.687	3.64	9.517	6.09
Serum Pool 3	312.483	6.335	2.03	18.238	5.84	19.307	6.18
Control 1	38.706	1.972	5.09	1.515	3.91	2.487	6.43
Control 2	206.470	7.305	3.54	11.203	5.43	13.374	6.48
Control 3	315.418	6.032	1.91	15.912	5.04	17.017	5.40

Limit of Blank (LoB)

The LoB for the Cortisol assay is 2.5 ng/mL.

Measuring Range

2.5-600 ng/mL (defined by the limit of blank and the maximum of the master curve). Values below the limit of blank are reported as <2.5 ng/mL. Values above the measuring range are reported as >600 ng/mL.

Recovery

Known concentrations of Cortisol were added to normal samples. The concentration of Cortisol was determined using the Cortisol assay, and the resulting percent recovery was calculated. The recovery should be within 90% -110%.

Sample	Added (ng/mL)	Observed (ng/mL)	%Recovery
S1	-	32.412	-
	18.47	49.922	94.80
	252.30	287.488	101.10
S2	-	103.612	-
	18.47	121.740	98.15

	252.30	348.595	97.10
	-	295.857	-
S3	18.47	314.734	102.20
	252.30	537.687	95.85

Method Comparison
A total of 123 serum samples in the range of 11.18 to 589.18 ng/mL were tested by the Cortisol assay (y) and a commercially available immunoassay (x). The data from the resulting linear regressions are summarized as: y=0.978x+5.0268, r^2=0.975. And a total of 136 urine samples in the range of 14.81 to 541.69 μ g/24 hours were tested by the Cortisol assay (y) and a commercially available immunoassay (x). The data from the resulting linear regressions are summarized as: y=0.981x+4.0893, r^2=0.966.

Analytical Specificity
The specificity of the assay was obtained by adding Testosterone (100 ng/mL), Progesterone (100 ng/mL) and Androstenedione (100 ng/mL) to two serum samples containing 52 and 350 ng/mL of Cortisol, respectively. No interference was found.

Endogenous Interference
Substances up to the following concentrations did not interfere with the assay:
- Bilirubin 60 mg/dL
- Hemoglobin 1900 mg/dL
- Triglyceride 2700 mg/dL

REFERENCES:

1. Scott, E. (2011). Cortisol and stress: How to stay healthy. Annals of the New York Academy of Sciences, 1024(1), 138-146.
2. Chyun YS, Kream BE, Raisz LG (February 1984). "Cortisol decreases bone formation by inhibiting periosteal cell proliferation". Endocrinology. 114 (2): 477–80.
3. Marieb, E. N., & Hoehn, K. (2007). Human anatomy & physiology. Pearson Education.
4. Gatti R, Antonelli G, Prearo M, et al. Cortisol assays and diagnostic laboratory procedures in human biological fluids. Clin Biochem 2009; 42(12):1205-1217.
5. Dallman, M. F., & Yates, F. E. (1969). Dynamic asymmetries in the corticosteroid feedback path and distribution-metabolism-binding elements of the adrenocortical system. Annals of the New York Academy of Sciences, 156(2), 696-721.
6. Keller-Wood, M. E., & Dallman, M. F. (1984). Corticosteroid inhibition of ACTH secretion. Endocrine reviews, 5(1), 1-24.
7. Davies E, Kenyon CJ, Fraser R (June 1985). "The role of calcium ions in the mechanism of ACTH stimulation of cortisol synthesis". Steroids. 45 (6): 551–60.
8. Plotsky PM, Otto S, Sapolsky RM (September 1986). "Inhibition of immunoreactive corticotropin-releasing factor secretion into the hypophysial-portal circulation by delayed glucocorticoid feedback". Endocrinology. 119 (3): 1126–30.
9. Turpeinen U, Hämäläinen E. Determination of cortisol in serum, saliva and urine. Best Practice & Research Clinical Endocrinology & Metabolism 2013; 27(6):795-801.
10. Gold, E. M. (1979). The Cushing syndromes: changing views of diagnosis and treatment. Annals of Internal Medicine, 90(5), 829-844.
11. Hsu, T. H. (1983). The pituitary-adrenal axis: clinical considerations. J Clin Immunoassay, 6, 277-87.

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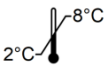
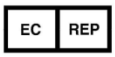
SYMBOLS EXPLANATIONS

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