Snibe Diagnostic

CE

REF 130205002M: 100 tests
130605002M: 50 tests

MAGLUMI® Insulin (CLIA)

INTENDED USE

The kit is an *in vitro* chemiluminescence immunoassay for the quantitative determination of insulin in human serum 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

Insulin is a peptide hormone produced by beta cells of the pancreatic islets. Insulin consists of two polypeptide chains, the A- and B- chains, linked together by disulfide bonds. It is however first synthesized as a single polypeptide called preproinsulin in pancreatic β-cells. Preproinsulin contains a 24-residue signal peptide which directs the nascent polypeptide chain to the rough endoplasmic reticulum (RER). The signal peptide is cleaved as the polypeptide is translocated into lumen of the RER, forming proinsulin. In the RER the proinsulin folds into the correct conformation and 3 disulfide bonds are formed. About 5–10 min after its assembly in the endoplasmic reticulum, proinsulin is transported to the trans-Golgi network (TGN) where immature granules are formed[1]. Proinsulin undergoes maturation into active insulin through the action of cellular endopeptidases known as prohormone convertases (PC1 and PC2), as well as the exoprotease carboxypeptidase E[2].

Insulin regulates the metabolism of carbohydrates, fats and protein by promoting the absorption of, especially, glucose from the blood into fat, liver and skeletal muscle cells. In these tissues the absorbed glucose is converted into either glycogen via glycogenesis or fats (triglycerides) via lipogenesis, or, in the case of the liver, into both. Glucose production (and excretion into the blood) by the liver is strongly inhibited by high concentrations of insulin in the blood. Circulating insulin also affects the synthesis of proteins in a wide variety of tissues. It is therefore an anabolic hormone, promoting the conversion of small molecules in the blood into large molecules inside the cells. Low insulin levels in the blood have the opposite effect by promoting widespread catabolism[3,4].

There are several conditions in which insulin disturbance is pathologic. A too low concentration of free, biologically active insulin can lead to the development of diabetes mellitus, which include two types: Type1 diabetes indicates autoimmune-mediated destruction of insulin-producing β-cells in the pancreas, resulting in absolute insulin deficiency; Type2 diabetes indicates either inadequate insulin production by the β-cells or insulin resistance or both because of reasons not completely understood. It is likely that there is genetic susceptibility to develop Type 2 diabetes under certain environmental conditions. In addition, various pathological conditions could occur such as insulinoma (a tumor of pancreatic β-cells producing excess insulin or reactive hypoglycemia), metabolic syndrome like hypertension, obesity and cardiovascular disease etc[5-8].

PRINCIPLE OF THE TEST

The Insulin assay is a sandwich chemiluminescence immunoassay.

The sample (or calibrator/control, if applicable), buffer, magnetic microbeads coated with anti-Insulin monoclonal antibody and ABEI labeled with anti-Insulin monoclonal antibody are mixed thoroughly and incubated, formed sandwich of immuno-complexes. After precipitation in a magnetic field, the supernatant is decanted and then a wash cycle is performed. Subsequently, the Starter1+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 Insulin present in the sample (or calibrator/control, if applicable).

KIT COMPONENTS

Material Provided

Components	**Contents**	100 tests (REF:130205002M)	50 tests (REF:130605002M)
Magnetic Microbeads	Magnetic microbeads coated with anti-Insulin monoclonal antibody, containing BSA, NaN_3 (<0.1%).	2.5 mL	2.0 mL
Calibrator Low	Insulin antigen, containing BSA, NaN_3 (<0.1%).	2.5 mL	2.0 mL
Calibrator High	Insulin antigen, containing BSA, NaN_3 (<0.1%).	2.5 mL	2.0 mL
Buffer	Containing BSA, NaN_3 (<0.1%).	10.5 mL	7.0 mL
ABEI Label	Anti-Insulin monoclonal antibody labeled with ABEI, containing BSA, NaN_3 (<0.1%).	10.5 mL	7.0 mL
Internal Quality Control	Containing BSA and Insulin antigen, 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 traceable to the WHO 1st International Standard 83/500.

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 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 ***Insulin (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

- 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 matter 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 only 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 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.
- Specimens removed from the separator, red blood cells or clot may be stored up to 12 hours at 2-8°C, and stored up to 6 months frozen at -20°C or colder.
- Before shipping specimens, it is recommended that specimens be removed from the serum 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 insulin 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. The recommended dilution is 1:19. 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 found for Insulin concentrations up to 2,000 µIU/mL.

LIMITATION

- 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 the patient's clinical picture and other diagnostic procedures.
- 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 serum concentrations may occasionally influence results.
- Hemolyzed samples should not be used, since enzymatic degradation of insulin may occur and result in lower assay values.
- Samples from patients treated with bovine, porcine or human insulin sometimes contain anti-insulin antibodies which can affect the test results.

RESULTS

Calculation of Results

The analyzer automatically calculates the Insulin 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 µIU/mL. For further information please refer to the corresponding Analyzer Operating Instructions.

Interpretation of Results

The expected range for the Insulin assay was obtained by testing 116 apparently healthy individuals in China, and gave the following expected value: 4.03-23.46 µIU/mL (before meal) (2.5^{th}-97.5^{th} percentiles)
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 Insulin assay was determined as described in the CLSI EP5-A2. 3 human serum pools and 2 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(µIU/mL) (N=80)	Within-Run		Between-Run		Total	
		SD(µIU/mL)	%CV	SD(µIU/mL)	%CV	SD(µIU/mL)	%CV
Serum Pool 1	16.546	0.827	5.00	0.258	1.56	0.867	5.24
Serum Pool 2	45.804	1.262	2.76	1.375	3.00	1.866	4.07
Serum Pool 3	142.136	2.670	1.88	1.492	1.05	3.059	2.15
Control 1	4.165	0.227	5.45	0.129	3.10	0.261	6.27
Control 2	25.664	1.053	4.10	0.331	1.29	1.103	4.30

Limit of Blank (LoB)

The LoB for the Insulin assay is 0.3 µIU/mL.

Limit of Detection (LoD)

The LoD for the Insulin assay is 0.5 µIU/mL.

Measuring Range

0.3-200 µIU/mL (defined by the limit of blank and the maximum of the master curve). Values below the limit of blank are reported as <0.3 µIU/mL. Values above the measuring range are reported as >200 µIU/mL.

Linearity

The assay is linear between 0.5 µIU/mL and 200 µIU/mL based on a study performed with guidance from CLSI EP6-A. Nine equally distributed levels of samples were prepared by spiking a serum sample containing insulin 220 µIU/mL with a serum sample depleted of insulin (0.0

μIU/mL).The Mean sample recovery ranged between 90.0% to 110.0%.

Method Comparison

A total of 100 samples in the range of 3.04 to 188.46 μIU/mL were tested by the Insulin assay (y) and a commercially available immunoassay (x). The data from the resulting linear regressions are summarized as: y = 0.964x+0.819, r^2 = 0.995.

Analytical Specificity

Recovery studies were performed to compare serum containing the following compounds at the indicated concentrations with serum samples. The Cross-reactivity of the assay was below:

Cross-Reactant	Concentration	%Cross-reactivity
Proinsulin	1000ng/mL	ND
Human C-peptide	200 ng/mL	ND
Insulin-like growth factor I (IGF-I)	200 ng/mL	ND
Bovine insulin	0.5 ng/mL	80
Porcine insulin	0.5 ng/mL	100

Note: ND = not detectable

Endogenous Interference

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

- Bilirubin 20 mg/dL
- Triglyceride 500 mg/dL
- Hemoglobin 3000 mg/dL

References:

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9. chroff RW, Foon KA, Beatty SM, et al. Human anti-murine immunoglobulin responses in patients receiving monoclonal antibody therapy. Cancer Res 1985; 45(2):879-885.
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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