Snibe Diagnostic

CE

REF 130201009M: 100 tests
130601009M: 50 tests

MAGLUMI® CA 125 (CLIA)

INTENDED USE

The kit is an *in vitro* chemiluminescence immunoassay for the quantitative determination of Cancer Antigen 125 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

CA-125 (cancer antigen 125, carcinoma antigen 125, or carbohydrate antigen 125) also known as mucin 16 or MUC16 is a protein that in humans is encoded by the MUC16 gene[1-2]. MUC16 is a member of the mucin family glycoproteins. Mucin 16 is a membrane associated mucin that possesses a single transmembrane domain. A unique property of MUC16 is its large size. MUC16 is more than twice as long as MUC1 and MUC4 and contains about 22,000 amino acids, making it the largest membrane-associated mucin[3-5].

MUC16 is composed of three different domains: an N-terminal domain, a central tandem repeat region, and a carboxyl-terminal domain. The N-terminal and tandem repeat domains are entirely extracellular and highly O-glycosylated. In particular, the N-terminal domain consists of 12,070 amino acids rich in serine/threonine residues and has been reported to contain the major O-glycosylation known to be present in CA 125. The MUC16 protein back bone is composed of tandem repeat region, which has more than 60 repeat domains of 156 amino acids each. Though not all individually similar, most of the repeat units, which, such as any mucins, are rich in serine, threonine and proline residues, recur more than once inside the sequence. In the tandem repeat domain of MUC16 there is also a small cysteine ring region on which are thought to be present the epitopes for known anti-CA 125 antibodies[6-7].

CA-125 is the most frequently used biomarker for ovarian cancer detection. Medical societies including American Congress of Obstetricians and Gynecologists recommend against women with average risk of ovarian cancer having routine CA-125 screening or other screening for this cancer. Reasons for this include evidence that ambiguous test results are more likely to lead to further invasive, harmful, and unnecessary health care than they are likely to detect ovarian cancer in women who are at average risk of developing it[8-9]. Around 90% of women with advanced ovarian cancer have elevated levels of CA-125 in their blood serum, making CA-125 a useful tool for detecting ovarian cancer after the onset of symptoms[10]. Monitoring CA-125 blood serum levels is also useful for determining how ovarian cancer is responding to treatment (with the duration of disease-free survival correlating with the rate of fall of CA-125) and for predicting a patient's prognosis after treatment. This is because the persistence of high levels of CA-125 during therapy is associated with poor survival rates in patients. Also, an increase in CA-125 levels within individuals in a remission is a strong predictor of the recurrence of ovarian cancer[11-13].

PRINCIPLE OF THE TEST

The CA 125 assay is a sandwich chemiluminescence immunoassay.

The sample (or calibrator/control, if applicable), buffer, magnetic microbeads coated with anti-CA 125 monoclonal antibody and ABEI labeled with anti-CA 125 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 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 CA 125 present in the sample (or calibrator/control, if applicable).

KIT COMPONENTS

Material Provided

Components	Contents	100 tests (REF: 130201009M)	50 tests (REF: 130601009M)
Magnetic Microbeads	Magnetic microbeads coated with anti-CA 125 monoclonal antibody, containing BSA, NaN_3 (<0.1%).	2.5 mL	2.0 mL
Calibrator Low	CA 125 antigen, containing BSA, NaN_3 (<0.1%).	3.0 mL	2.0 mL
Calibrator High	CA 125 antigen, containing BSA, NaN_3 (<0.1%).	3.0 mL	2.0 mL
Buffer	Containing BSA, NaN_3 (<0.1%).	6.5 mL	4.5 mL
ABEI Label	Anti-CA 125 monoclonal antibody labeled with ABEI, containing BSA, NaN_3 (<0.1%).	6.5 mL	4.5 mL
Diluent	Containing bovine serum, NaN_3 (<0.1%).	25.0 mL	15.0 mL
Internal Quality Control	CA 125 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 traceable to the SNIBE internal reference substance.

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 ***CA 125 (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 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 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, cells or clot may be stored up to 5 days at 2-8°C, and stored up to 3 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 CA 125 is 80 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

Samples with concentrations above the measuring range can be diluted.
After manual dilution, multiply the result by the dilution factor. After dilution by the analyzers, the analyzer software automatically takes the dilution into account when calculating the sample concentration.

The automatic sample dilution is available after dilution settings are done in the MAGLUMI and Biolumi series Fully-auto chemiluminescence immunoassay analyzer. Please refer to the corresponding Analyzer Operating Instructions.

High-Dose Hook

No high-dose hook effect was found for CA 125 concentrations up to 5,000 U/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 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.

RESULTS

Calculation of Results

The analyzer automatically calculates the CA 125 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 U/mL. For further information please refer to the operating instructions of MAGLUMI series Fully-auto chemiluminescence immunoassay analyzer.

Interpretation of Results

The expected range for the CA 125 assay was obtained by testing 246 apparently healthy individuals in China, and gave the following expected value:

<35 U/mL (95^{th} percentile).

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

PERFORMANCE CHARACTERISTICS

Precision

Precision for the CA 125 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(U/mL) (N=80)	Within-Run		Between-Run		Total	
		SD(U/mL)	%CV	SD(U/mL)	%CV	SD(U/mL)	%CV
Serum Pool 1	19.990	0.798	3.99	1.259	6.30	1.491	7.46
Serum Pool 2	40.191	1.576	3.92	1.524	3.79	2.192	5.45
Serum Pool 3	499.477	10.016	2.01	7.445	1.49	12.480	2.50
Control 1	37.338	1.721	4.61	1.601	4.29	2.350	6.29
Control 2	121.066	3.312	2.74	2.911	2.40	4.409	3.64
Control 3	254.023	5.954	2.34	7.352	2.89	9.461	3.72

Limit of Blank (LoB)

The LoB for the CA 125 assay is 0.5 U/mL.

Limit of Detection (LoD)

The LoD for the CA 125 assay is 1.0 U/mL.

Measuring Range

0.5-1200 U/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 U/mL. Values above the measuring range are reported as >1200 U/mL.

Linearity

The assay is linear between 1.0 U/mL and 1200 U/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 CA 125 1300 U/mL with a serum sample depleted of CA 125 (0.0 U/mL). The mean sample recovery ranged between 90% to 110%.

Method Comparison

A total of 300 samples in the range of 0.896 and 1046.626 U/mL were tested by the CA 125 assay (y) and a commercially available immunoassay (x). The data from the resulting linear regressions are summarized as: $y=0.938x+1.8457$, $r^2=0.9820$.

Endogenous Interference

Controlled studies of potentially interfering substances or conditions showed that the assay performance was not affected by concentrations of bilirubin up to 66 mg/dL, hemoglobin up to 3200 mg/dL, triglycerides up to 2000mg/dL or RF up to 1200 IU/mL.

Drug Interference

Controlled studies of potentially interfering substances showed that the assay performance was not affected by the following common antitumor drugs.

Interference	Concentration
Cisplatin	165 µg/mL
Bleomycin	30 µg/mL
Carboplatin	500 µg/mL
Fluorouracil	400 µg/mL
Cytarabine	30 µg/mL
Methotrexate	909 µg/mL

mitomycin-C	100 µg/mL
Paclitaxel	67 µg/mL
Vinblastine sulfate	500 µg/mL
Doxorubicin hydrochloride	40 µg/mL
Tamoxifen	0.0228 µg/mL
Cyclophosphamide	1000 µg/mL

Analytical Specificity
The specificity data of the assay was obtained by adding CA15-3 (800 U/mL), CA19-9 (800 U/mL) and CA72-4 (800 U/mL) to serum samples at the indicated concentrations. No interference was found.

REFERENCES

1. Yin BW, Lloyd KO (Jul 2001). "Molecular cloning of the CA 125 ovarian cancer antigen: identification as a new mucin, MUC16". The Journal of Biological Chemistry. 276 (29): 27371–5.
2. Yin BW, Dnistrian A, Lloyd KO (Apr 2002). "Ovarian cancer antigen CA 125 is encoded by the MUC16 mucin gene". International Journal of Cancer. Journal International Du Cancer. 98 (5): 737–40.
3. Duraisamy S, Ramasamy S, Kharbanda S, Kufe D (May 2006). "Distinct evolution of the human carcinoma-associated transmembrane mucins, MUC1, MUC4 AND MUC16". Gene. 373: 28–34.
4. Gipson IK (Oct 2007). "The ocular surface: the challenge to enable and protect vision: the Friedenwald lecture". Investigative Ophthalmology & Visual Science. 48 (10): 4390; 4391–8.
5. Gniewek P, Kolinski A (Jan 2012). "Coarse-grained modeling of mucus barrier properties". Biophysical Journal. 102 (2): 195–200.
6. O'Brien TJ, Beard JB, Underwood LJ, Dennis RA, Santin AD, York L (Nov–Dec 2001). "The CA 125 gene: an extracellular superstructure dominated by repeat sequences". Tumour Biology. 22 (6): 348–66.
7. Hollingsworth MA, Swanson BJ (Jan 2004). "Mucins in cancer: protection and control of the cell surface". Nature Reviews. Cancer. 4 (1): 45–60.
8. Suh KS, Park SW, Castro A, Patel H, Blake P, Liang M, Goy A (Nov 2010). "Ovarian cancer biomarkers for molecular biosensors and translational medicine". Expert Review of Molecular Diagnostics. 10 (8): 1069–83.
9. Partridge E, Kreimer AR, Greenlee RT, Williams C, Xu JL, Church TR, Kessel B, Johnson CC, Weissfeld JL, Isaacs C, Andriole GL, Ogden S, Ragard LR, Buys SS (Apr 2009). "Results from four rounds of ovarian cancer screening in a randomized trial". Obstetrics and Gynecology. 113 (4): 775–82.
10. Partridge E, Kreimer AR, Greenlee RT, Williams C, Xu JL, Church TR, Kessel B, Johnson CC, Weissfeld JL, Isaacs C, Andriole GL, Ogden S, Ragard LR, Buys SS (Apr 2009). "Results from four rounds of ovarian cancer screening in a randomized trial". Obstetrics and Gynecology. 113 (4): 775–82.
11. Partridge E, Kreimer AR, Greenlee RT, Williams C, Xu JL, Church TR, Kessel B, Johnson CC, Weissfeld JL, Isaacs C, Andriole GL, Ogden S, Ragard LR, Buys SS (Apr 2009). "Results from four rounds of ovarian cancer screening in a randomized trial". Obstetrics and Gynecology. 113 (4): 775–82.
12. Göcze P, Vahrson H (Apr 1993). "[Ovarian carcinoma antigen (CA 125) and ovarian cancer (clinical follow-up and prognostic studies)]". Orvosi Hetilap (in Hungarian). 134 (17): 915–8.
13. Santillan A, Garg R, Zahurak ML, Gardner GJ, Giuntoli RL, Armstrong DK, Bristow RE (Dec 2005). "Risk of epithelial ovarian cancer recurrence in patients with rising serum CA-125 levels within the normal range". Journal of Clinical Oncology. 23 (36): 9338–43.

Shenzhen New Industries Biomedical Engineering Co., Ltd.
No.23, Jinxiu East Road, Pingshan District, 518122 Shenzhen, P.R. China
Tel: +86-755-21536601 Fax: +86-755-28292740

EC REP **Shanghai International Holding Corp. GmbH (Europe)**
Eiffestrasse 80, 20537 Hamburg, Germany
Tel: +49-40-2513175 Fax: +49-40-255726

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