Snibe Diagnostic

CE REF 130202007M: 100 tests
130602007M: 50 tests

MAGLUMI® Estradiol (CLIA)

INTENDED USE

The kit is an *in vitro* chemiluminescence immunoassay for the quantitative determination of Estradiol 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

Estradiol (E2), also spelled oestradiol, is a steroid, an estrogen, and the primary female sex hormone. It is named for and is important in the regulation of the estrous and menstrual female reproductive cycles. Estradiol is essential for the development and maintenance of female reproductive tissues such as the breasts, uterus, and vagina during puberty, adulthood, and pregnancy[1], but it also has important effects in many other tissues, including bone, fat, skin, liver, and the brain. While estrogen levels in men are lower compared to women, estrogens have essential functions in men, as well. It is found in most vertebrates and crustaceans, insects, fish, and other animal species[2-3]. Estradiol is produced especially within the follicles of the female ovaries, but also in other endocrine (i.e., hormone-producing) and nonendocrine tissues (e.g., including fat, liver, adrenal, breast, and neural tissues). Estradiol is biosynthesized from cholesterol through a series of chemical intermediates[4].

Levels of estradiol in premenopausal women are highly variable throughout the menstrual cycle and reference ranges widely vary from source to source[5]. Estradiol levels are minimal and according to most laboratories range from 20 to 80 pg/mL during the early to mid follicular phase (or the first week of the menstrual cycle, also known as menses)[6-7]. Levels of estradiol gradually increase during this time and through the mid to late follicular phase (or the second week of the menstrual cycle) until the pre-ovulatory phase. At the time of pre-ovulation (a period of about 24 to 48 hours), estradiol levels briefly surge and reach their highest concentrations of any other time during the menstrual cycle. Circulating levels are typically between 130 and 200 pg/mL at this time, but in some women may be as high as 300 to 400 pg/mL, and the upper limit of the reference range of some laboratories are even greater (for instance, 750 pg/mL)[5-6,8-10]. Following ovulation (or mid-cycle) and during the latter half of the menstrual cycle or the luteal phase, estradiol levels plateau and fluctuate between around 100 and 150 pg/mL during the early and mid luteal phase, and at the time of the late luteal phase, or a few days before menstruation, reach a low of around 40 pg/mL[5,7]. The mean integrated levels of estradiol during a full menstrual cycle have variously been reported by different sources as 80, 120, and 150 pg/mL. Although contradictory reports exist, one study found mean integrated estradiol levels of 150 pg/mL in younger women whereas mean integrated levels ranged from 50 to 120 pg/mL in older women[7,11].

PRINCIPLE OF THE TEST

The Estradiol assay is a competitive chemiluminescence immunoassay.

The sample (or calibrator/control, if applicable), ABEI labeled with anti-E2 monoclonal antibody, and magnetic microbeads coated with purified E2 antigen are mixed thoroughly and incubated, forming antibody-antigen 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 E2 present in the sample (or calibrator/control, if applicable).

KIT COMPONENTS

Material Provided

Component	Contents	100 tests (REF: 130202007M)	50 tests (REF: 130602007M)
Magnetic Microbeads	Magnetic microbeads coated with Estradiol antigen, containing BSA, NaN_3 (<0.1%).	2.5 mL	2.0 mL
Calibrator Low	Containing BSA and E2 antigen, NaN_3 (<0.1%).	3.0 mL	2.0 mL
Calibrator High	Containing BSA and E2 antigen, NaN_3 (<0.1%).	3.0 mL	2.0 mL
Buffer	Containing BSA, NaN_3 (<0.1%).	6.5 mL	4.0 mL
ABEI Label	Anti-E2 monoclonal antibody labeled with ABEI, containing BSA, NaN_3 (<0.1%).	6.5 mL	4.0 mL
Diluent	Containing BSA, NaN_3 (<0.1%).	25.0 mL	15.0 mL
Internal Quality Control	Containing BSA and E2 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 standardized against the USP Estradiol 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 2 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 ***Estradiol (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 serum 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 repeating freeze-thaw cycles. The serum sample can be only frozen and thawed one time. Specimens must be mixed thoroughly after thawing.
- 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 24 hours at 2-8°C. Freeze samples at or below -20°C if the sample is not assayed within 24 hours.
- 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 Estradiol 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

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 user software. Please refer to the corresponding Analyzer Operating Instructions.

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.
- 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 judgments.
- 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 Estradiol 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 pg/mL. For further information please refer to the corresponding Analyzer Operating Instructions.
Conversion factor: pg/mLx3.67=pmol/L.

Interpretation of Results

The expected ranges for the Estradiol assay were obtained by testing 373 females and 97 males from healthy individuals in China and gave the following expected values:
Males: <87 pg/mL (95^{th} percentile).
Females:

Phase	N	2.5^{th}-97.5^{th} percentiles (pg/mL)
Follicular phase	70	15-112
Preovulatory phase	72	136-251
Luteal phase	75	48-172
Post menopausal	77	10-66
Hormonal contraceptives	79	15-95

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 Estradiol assay was determined as described in the CLSI EP5-A2, 2 controls and 3 human serum pools 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
Serum Pool 1	51.306	3.512	6.84	2.592	5.05	4.365	8.51
Serum Pool 2	252.905	16.993	6.72	5.315	2.10	17.805	7.04
Serum Pool 3	2032.627	30.509	1.50	43.304	2.13	52.972	2.61
Control 1	74.746	3.946	5.28	1.818	2.43	4.344	5.81
Control 2	325.035	15.233	4.69	6.090	1.87	16.405	5.05

Limit of Blank (LoB)

The LoB for the Estradiol assay is 5.0 pg/mL.

Limit of Detection (LoD)

The LoD for the Estradiol assay is 12 pg/mL.

Measuring Range

5.0-6000 pg/mL (defined by the limit of blank and the maximum of the master curve). Values below the limit of blank are reported as <5.0 pg/mL. Values above the measuring range are reported as >6000 pg/mL.

Method Comparison

A total of 100 samples in the range of 20.26 to 5435.30 pg/mL were tested using the Estradiol assay (y) and a commercially available immunoassay (x). The data from the resulting linear regressions are summarized as: y=0.987x+11.62, r^2=0.988.

Analytical Specificity

The specificity of the assay was obtained by adding PROG (100 ng/mL), TEST (100 ng/mL), CORTISOL (300 ng/mL), and DHEA-S (50 ng/mL) to serum samples at the indicated concentrations. No interference was found.

Endogenous Interference

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

- Bilirubin 66 mg/dL

- Hemoglobin 1000 mg/dL
- Triglyceride 1000 mg/dL

Due to the risk of cross reactivity, this assay should not be used when monitoring Estradiol levels in patients being treated with Fulvestrant.

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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