IVD

ESTRADIOL (E2) CHEMILUMINESCENCE IMMUNOASSAY KIT

Catalog No. CL1105-2

INTENDED USE

The Autobio estradiol (E2) chemiluminescence immunoassay (CLIA) kit is intended for the quantitative determination of E2 concentration in human serum.

INTRODUCTION

Estradiol (E2) is a C18 steroid hormone with a phenolic A ring. This steroid hormone has a molecular weight of 272.4 daltons. It is the most potent natural estrogen, produced mainly by the ovary, placenta, and in smaller amounts by the adrenal cortex, and the male testes.

Estradiol (E2) is secreted into the blood stream where 98% of it circulates bound to sex hormone binding globulin (SHBG). To a lesser extent it is bound to other serum proteins such as albumin. Only a tiny fraction circulates as free hormone or in the conjugated form. Estrogenic activity is effected via Estradiol-receptor complexes which trigger the appropriate response at the nuclear level in the target sites. These sites include the follicles, uterus, breast, vagina, urethra, hypothalamus, pituitary and to a lesser extent the liver and skin.

In non-pregnany women with normal menstrual cycles, Estradiol secretion follows a cyclic, biphasic pattern with the highest concentration found immediately prior to ovulation. The rising Estradiol concentration is understood to exert a positive feedback influence at the level of the pituitary where it influences the secretion of the gonadotropins, follicle stimulating hormone (FSH), and luteinising hormone (LH), which are essential for follicular maturation and ovulation, respectively. Following ovulation, Estradiol levels fall rapidly until the luteal cells become active resulting in a secondary gentle rise and plateau of Estradiol in the luteal phase. During pregnancy, maternal serum Estradiol levels increase considerably, towel above the pre-ovulatory peak levels and high levels are sustained throughout pregnancy.

Serum Estradiol measurements are a valuable index in evaluationg a variety of menstrual dysfunctions such as precocious or delayed puberty in girls and primary and secondary amenorrhea and menopause. Estradiol levels have been reported to be increased in patients with feminizing syndromes, gynaecomastia and testicular tumors.

In cases of infertility, serum Estradiol measurements are useful for monitoring induction of ovulation following treatment with, for example, clomiphene citrate, LH-releasing hormone (LH-RH), or exogenous gonadotropins. During ovarian hyperstimulation usually monitored daily for optimal timing of human chorionic gonadotropin (HCG) adminstration and oocyte collection.

PRINCIPLE OF THE TEST

The E2 CLIA test is based on the principle of competitive binding between E2 in the test specimen and E2-HRP conjugate for a constant amount of rabbit anti-Estradiol. In the incubation, goat anti-rabbit IgG-coated wells are incubated with E2 standards, patient samples, E2-HRP conjugate and rabbit anti-Estradiol reagent at room temperature. During the incubation, a fixed amount of HRP-labeled E2 competes with the endogenous E2 in the standard, samples for a fixed number of binding sites of the specific E2 antibody. Thus, the amount of E2 peroxidase conjugate immunologically bound to the well progressively decreases as the concentration of E2 in the specimen increases. Unbound E2 peroxidase conjugate is then removed and the wells washed. A chemiluminescence reaction is developed when the CLIA substrate is mixed with the antibody bound estradiol-horseradish peroxidase enzyme conjugate. The Related Light Unit (RLU) is proportional to the amount of enzyme present and is inversely related to the amount of unlabeled E2 in the sample. By reference to a series of E2 standards assayed in the same way, the concentration of E2 in the unknown sample is quantified.

MATERIALS PROVIDED

1. Antibody Coated Microtiter Plate: Microplate coated with goat anti-rabbit IgG (1 plate, 48 wells/96wells)
2. Enzyme Conjugate Reagent: Horseradish peroxidase (HRP) labeled estradiol (E2) in Stabilizing Buffer (1 vial, 5.5ml/11.0 ml)
3. Anti-E2 Reagent: Rabbit polyclonal antibodies to E2 (anti-E2 PAb) in Stabilizing Buffer (1 vial, 2.8ml/5.5 ml)
4. Reference Standards: 0, 30, 100, 300, and 1000 pg/ml E2 in Stabilizing Buffer (5 vials, 0.3ml/ea)
5. Substrate A: (1 vial, 1.8ml/3.5ml)

6. Substrate B: (1 vial, 1.8ml/3.5ml)
7. PBS-T Powder: PBS-Tween (1bag, 5g)

MATERIALS NOT PROVIDED

The following materials are required but not provided in the kit.

1. Precision pipettes and tips, 0.025ml, 0.05ml, 0. 1ml
2. Distilled water.
3. Vortex mixer
4. Absorbent paper or paper towel
5. Graph paper
6. Luminometer

STORAGE OF TEST KIT AND INSTRUMENTATION

1. Unopened test kits should be stored at 2 ～ 8℃ upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit. Refer to the package label for the expiration date.
2. Opened test kits will remain stable until the expiration date shown, provided it is stored as prescribed above.

SPECIMEN COLLECTION AND PREPARATION

1. Blood should be drawn using standard venipuncture techniques and the serum should be separated from the red blood cells as soon as practical. Avoid grossly hemolytic, lipemic or turbid samples.
2. Plasma samples collected in tubes containing EDTA, heparin, or oxalate may interfere with test procedures and should be avoided.
3. Specimens should be capped and may be stored up to 24 hours at 2 ～ 8℃, prior to assaying. Specimens held for a longer time can be frozen at -20℃. Thawed samples must be mixed prior to testing. Multiple freeze-thaw cycles should be avoided.

PRECAUTIONS AND WARNINGS

1. For *in vitro* diagnostic use only.
2. Handling of reagents, serum specimens should be in accordance with local safety procedures.
3. The reference satandards contain human source components, which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody. All animal products and derivatives have been collected from healthy animals. Bovine components originate from countries where BSE has not been reported. Nevertheless, the satandards and components containing animal substances should be treated as potentially infectious.
4. Avoid any skin contact with all reagents.
5. Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.

REAGENT PREPARATION

1. All reagents should be brought to room temperature (18 ～ 25℃) before use. All reagents should be mixed by gently inverting or swirling prior to use. Do not induce foaming.
2. Prepare Wash Solution: add 1 bag of PBS-T powder to 500ml of distilled water, and mix well with a magnetic stirrer. The Wash Solution is stable at room temperature for 2 months.

IMPORTANT NOTES

1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated RLU values.
2. It is recommended that no more than 32 wells be used for each assay run, if manual pipetting is used, since pipetting of all standards, specimens and controls should be completed within 5 minutes. A full plate of 96 wells may be used if automated pipetting is available.
3. Duplication of all standards and specimens, although not required, is recommended.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder. Dispense 25μl of reference standards, specimens, and controls into appropriate wells.
2. Dispense 100μl of Enzyme Conjugate Reagent into each well.

3. Dispense 50μl of Anti-E2 Reagent into each well. Mix gently for 30 seconds.
4. Place on a vortex mixer for 60 minutes at room temperature.
5. At the end of the incubation, remove the incubation mixture by emptying the plate content into a waste container. Rinse and empty the microtiter plate 5 times with washing buffer. Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets. The volume of each well is about 300μl.
6. Dispense 25μl of substrate A, then 25μl of substrate B into each well. Gently mix for 10 seconds.
7. Incubate at room temperature in the dark for 10 minutes without shaking, then read the RLU values with a Luminolmeter.

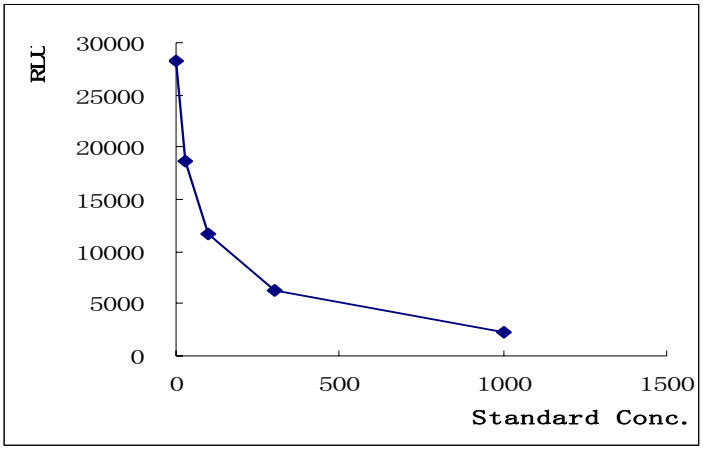
CALCULATION OF RESULTS

1. Calculate the mean value from any duplicate reagents. Where appropriate, the mean values should be used for plotting.
2. On linear graph paper plot the RLU (ordinate) for each Reference Standard against the corresponding concentration of E2 in pg/ml (abscissa) and draw a calibration curve through the reference standard points by connecting the plotted points with straight lines.
3. Read the concentration for each control and sample by interpolating on the calibration curve.
4. Computer assisted data reduction will simplify these calculations. If automatic result processing is used, a 4-parameter logistic function curve fitting is recommended.

EXAMPLE OF STANDARD CURVE

A typical calibration curve shown below is for the purpose of illustration only, and should never be used instead of the real time calibration curve.

E2 (pg/ml)	RLU
0	28322.7
30	18681.4
100	11709.2
300	6283.7
1000	2279.3



EXPECTED VALUES

Each laboratory must establish its own normal ranges based on patient population. The results provided below are based on randomly selected clinical laboratory samples:

estradiol (pg/ml)

	Stage		**Normal Range**
Male			< 60
Female	Postmenopausal phase		< 18
	ovulating	early follicular	30-100
		late follicular	100-400
		luteal phase	60-150
	Pregnant, normal		Up to 35,000
	Prepuberal children, normal		< 10

PERFORMANCE

A. Sensitivity

The lower detection limit is calculated from the standard curve by identifying the concentration corresponding to the mean RLU of standard diluent (based on 10 replicate analyses) subtract 2 SD. Therefore, the sensitivity of the Autobio E2 CLIA kit is not higher than 10pg/ml.

B. Specificity

No interference was detected with the performance of Autobio E2 CLIA upon addition of massive amounts of the following substances to a human serum pool.

Interferents	**Concentration**
Testosterone	20ng/ml
Progesterone	100ng/ml

C. Precision

a. Intra-assay Precision

Intra-assay precision was determined by assaying 20 replicates of each control sera.

Serum	Number	Mean	SD	CV (%)
Low titer	20	88.72	5.01	5.65
High titer	20	543.93	18.42	3.39

b. Inter-assay Precision

Inter-assay precision was determined by assaying duplicates of each control sera in 10 separate runs.

Serum	Number	Mean	SD	CV (%)
Low titer	10	81.16	3.61	4.45
High titer	10	526.86	13.15	2.50

D. Accuracy

For samples in the range of 10pg/ml to 1000pg/ml, the relationship between the Autobio E2 CLIA and the Beckman Access 2:180® E2 assay, is described by the equation below:

Method	**No. of Specimens**	**Linear Equation**	**Correlation Coefficient**
Beckman Access 2®	180	$y = 0.9312x + 0.089$	0.965

LIMITATIONS

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert and with adherence to good laboratory practice.
2. Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference thus anomalous values may be observed. Additional information may be required for diagnosis.
3. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
4. For diagnostic purposes, the results obtained from this assay should always be used in combination with the clinical examination, patient medical history, and other findings.

QUALITY CONTROL

Good laboratory practice requires that quality control specimens be run with each calibration curve to verify assay performance. To assure proper performance, a statistically significant number of controls should be assayed to establish mean values and acceptable ranges. Controls containing sodium azide should not be used.

SYMBOLS

	**BATCH CODE**
	**USE BY**
	**MANUFACTURER**
	**CONTAINS SUFFICIENT FOR <n> TESTS**

IVD	***IN VITRO* DIAGNOSTIC MEDICAL DEVICE**
8 °C 2 °C	**TEMPERATURE LIMITATION**
REF	**CATALOGUE NUMBER**
	CONSULT INSTRUCTIONS FOR USE

REFERENCES

1. Tsang, B.K., etc, Steroid biosyntheses by isolated human ovarian follicular cells in vitro, J. Clin. Endocrinol. Metab. 51:1407-1411(1980)
2. Gore-Langton, etc. Follicular steroidogenesis and its control. In: The Physiology of Reproduction, Ed.: Knobil, E., and Neill, J. et al., pp. 331-385. Raven Press, New York (1988)
3. Hall, P. F., Testicular Steroid Synthesis: Organization and Regulation. In: The Physiology of Reproduction, Ed.: Knobil, E., and Neill, J. et al., pp. 975-998. Raven Press, New York (1988)
4. Siiteri, P.K. Murai, etc. The serum transport of steroid hormones, Rec. Prog. Horm. Res. 38:457-510(1982)
5. Martin, B., Rotten, D., etc. Binding of steroids by proteins in follicular fluid of the human ovary, J. Clin. Endicrinol. Metab. 35:443-447(1981)

For order and inquires, please contact

AUTOBIO DIAGNOSTICS CO., LTD.
ADD: No.87 Jingbei Yi Road, National Eco & Tech Development Area,
Zhengzhou , China 450016
Tel: +86-371-67985313 Fax: +86-371-67985804
Web: www.autobio.com.cn