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Labor Diagnostika Nord GmbH & Co. KG

Instructions for use

Estrone ELISA

REF FR E-2300

96

+2 +8 °C

IVD

CE

Estrone ELISA

INTENDED USE

For the direct quantitative determination of Estrone by enzyme immunoassay in human serum.
For *in vitro* diagnostic use only.

PRINCIPLE OF THE TEST

The principle of the following enzyme immunoassay test follows the typical competitive binding scenario. Competition occurs between an unlabeled antigen (present in standards, controls and patient samples) and an enzyme-labelled antigen (conjugate) for a limited number of antibody binding sites on the microwell plate. The washing and decanting procedures remove unbound materials. After the washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stopping solution. The absorbance is measured on a microtiter plate reader. The intensity of the colour formed is inversely proportional to the concentration of estrone in the sample. A set of standards is used to plot a standard curve from which the amount of estrone in patient samples and controls can be directly read.

CLINICAL APPLICATIONS

Estrone is a steroid like estriol and estradiol, belonging to the class of estrogens. The estrogens are involved in the development of female sex organs and secondary sex characteristics. Before the ovum is fertilized the main action of the estrogens is on the growth and function of the reproductive tract in order to prepare it for the fertilized ovum.
During the follicular phase of the menstrual cycle the estrone level shows a slight increase. The production of estrone then increases markedly to peak at around day 13. The peak is of short duration and by day 16 of the cycle levels will be low. A second peak occurs at around day 21 of the cycle and if fertilization does not occur, then the production of estrone decreases.

PROCEDURAL CAUTIONS AND WARNINGS

- Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
- Control materials or serum pools should be included in every run at a high and low level for assessing the reliability of results.
- When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
- In order to reduce exposure to potentially harmful substances, gloves should be worn when handling kit reagents and human specimens.
- All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
- A calibrator curve must be established for every run.
- The controls should be included in every run and fall within established confidence limits.
- Improper procedural techniques, imprecise pipetting, incomplete washing as well as improper reagent storage may be indicated when assay values for the controls do not reflect established ranges.
- When reading the microplate, the presence of bubbles in the microwells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.
- The substrate solution (TMB) is sensitive to light and should remain colourless if properly stored. Instability or contamination may be indicated by the development of a blue colour, in which case it should not be used.
- When dispensing the substrate and stopping solution, do not use pipettes in which these liquids will come into contact with any metal parts.
- To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
- Do not mix various lot numbers of kit components within a test and do not use any component beyond the expiration date printed on the label.
- Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

LIMITATIONS

- All the reagents within the kit are calibrated for the direct determination of estrone in human serum. The kit is not calibrated for the determination of estrone in saliva, plasma or other specimens of human or animal origin.
- Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
- Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.
- Only calibrator A may be used to dilute any high serum samples. The use of any other reagent may lead to false results.
- The results obtained with this kit should never be used as the sole basis for a clinical diagnosis. For example, the occurrence of heterophilic antibodies in patients regularly exposed to animals or animal products has the potential of causing interferences in immunological tests. Consequently, the clinical diagnosis should include all aspects of a patient's background including the frequency of exposure to animals/products if false results are suspected.

SAFETY CAUTIONS AND WARNINGS
POTENTIAL BIOHAZARDOUS MATERIAL
Human serum that may be used in the preparation of the standards and controls has been tested and found to be non-reactive for Hepatitis B surface antigen and has also been tested for the presence of antibodies to HCV and Human Immunodeficiency Virus (HIV) and found to be negative. However no test method can offer complete assurance that HIV, HCV and Hepatitis B virus or any infectious agents are absent. The reagents should be considered a potential biohazard and handled with the same precautions as applied to any blood specimen.

CHEMICAL HAZARDS
Avoid contact with reagents containing TMB, hydrogen peroxide and sulfuric acid. If contacted with any of these reagents, wash with plenty of water. TMB is a suspected carcinogen.
SPECIMEN COLLECTION AND STORAGE
Approximately 0.2 ml of serum is required per duplicate determination. Collect 4-5 ml of blood into an appropriately labelled tube and allow it to clot. Centrifuge and carefully remove the serum layer. Store at 4°C for up to 24 hours or at -10°C or lower if the analyses are to be done at a later date. Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

SPECIMEN PRETREATMENT
This assay is a direct system; no specimen pretreatment is necessary.

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED
- Precision pipettes to dispense 50, 100, 150 and 300 µl
- Disposable pipette tips
- Distilled or deionized water
- Plate shaker
- Microwell plate reader with a filter set at 450nm and an upper OD limit of 3.0 or greater* (see assay procedure step 10).

REAGENTS PROVIDED

AA E-0030 WASH-CONC 10x **Wash Buffer Concentrate – X10**

Contents:	One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.
Volume:	50 mL/bottle
Storage:	Refrigerate at 2-8°C
Stability:	12 months or as indicated on label.
Preparation:	Dilute 1:10 in distilled or deionized water before use. If the whole plate is to be used dilute 50 mL of the wash buffer concentrate in 450 mL of water.

AA E-0055 SUBSTRATE **TMB Substrate** - Ready To Use.

Contents:	One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.
Volume:	16 mL/bottle
Storage:	Refrigerate at 2-8°C
Stability:	12 months or as indicated on label.

AA E-0080 STOP-SOLN **Stopping Solution** - Ready To Use.

Contents:	One vial containing 1M sulfuric acid.
Volume:	6 mL/bottle
Storage:	Refrigerate at 2-8°C
Stability:	12 months or as indicated on label.

Calibrators and **Controls**- Ready To Use.

Listed below are approximate concentrations, please refer to vial labels for exact concentrations:

Cat. no.	Symbol	Calibrator	Concentration	Volume/Vial
FR E-2301	STANDARD A	**Calibrator A**	0 pg/ml	2.0 ml
FR E-2302	STANDARD B	**Calibrator B**	15 pg/ml	0.5 ml
FR E-2303	STANDARD C	**Calibrator C**	50 pg/ml	0.5 ml
FR E-2304	STANDARD D	**Calibrator D**	200 pg/ml	0.5 ml
FR E-2305	STANDARD E	**Calibrator E**	800 pg/ml	0.5 ml
FR E-2306	STANDARD F	**Calibrator F**	2000 pg/ml	0.5 ml
FR E-2351	CONTROL 1	**Control 1**	Refer to vial labels for expected value and acceptable range!	0.5 ml
FR E-2352	CONTROL 2	**Control 2**		0.5 ml

Contents: Estrone in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of estrone.

Storage: Refrigerate at 2-8°C

Stability: 12 months in unopened vials or as indicated on label. Once opened, the standards should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

FR E-2313 ASSAY-BUFF **Assay Buffer** - Ready To Use.

Contents: One vial containing a protein-based buffer with a non-mercury preservative.

Volume: 15 mL/bottle

Storage: Refrigerate at 2-8°C

Stability: 12 months or as indicated on label.

FR E-2331 96 **Rabbit Anti-Estrone Antibody Coated Microwell Plate-Break Apart Wells** - Ready To Use.

Contents: One 96 well (12x8) polyclonal antibody-coated microwell plate in a resealable pouch with desiccant.

Storage: Refrigerate at 2-8°C

Stability: 12 months or as indicated on label.

FR E-2340 CONJUGATE-CONC 100x **Avidin-Horse Radish Peroxidase (HRP) Conjugate Concentrate - X100**

Contents: Avidin-HRP conjugate in a protein-based buffer with a non-mercury preservative.

Volume: 0.2 mL/vial

Storage: Refrigerate at 2-8°C

Stability: 12 months or as indicated on label.

FR E-2341 BIO-AB-CONC 100x **Estrone-Biotin Antibody Concentrate - X100**

Contents: Estrone-Biotin Antibody in a protein-based buffer with a non-mercury preservative

Volume: 0.2 mL/vial

Storage: Refrigerate at 2-8°C

Stability: 12 months or as indicated on label.

Preparation of Estrone-Biotin:Avidin-HRP conjugate working solution FR E-2340 + FR E-2341

Dilute both the estrone-biotin and avidin-HRP concentrates 1:100 into the same solution of assay buffer and mix thoroughly (eg. To a tube containing 2ml of assay buffer add 20µl of estrone-biotin and avidin-HRP conjugate concentrates).

ASSAY PROCEDURE

All reagents must reach room temperature before use. Calibrators, controls and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1.	Prepare **working solutions** of the **estrone-biotin: avidin-HRP conjugate** and **wash buffer**. After conjugate working solution is prepared, **it is essential that it be mixed and allowed to stand for <u>at least 15 minutes</u> prior to use.**
2.	Remove the required number of microwell strips. Reseal the bag and return any unused strips to the refrigerator.
3.	**Pipette 50 µl** of each **calibrator, control and specimen sample** into correspondingly labelled wells in duplicate.
4.	Pipette **100 µL** of the **conjugate working solution** into each well. *(We recommend using a multichannel pipette).*
5.	**Incubate** on a plate shaker (approximately 200 rpm) for **1 hour** at **room temperature**.
6.	Wash the wells **3 times** with prepared wash buffer (**300 µL**/well for each wash) and tap the plate firmly against absorbent paper to ensure that it is dry *(The use of a washer is recommended).*
7.	Pipette **150 µL** of **TMB substrate** into each well at timed intervals.
8.	Incubate the plate on a plate shaker at **room temperature** for **10-15** minutes. *(or until Calibrator A attains dark blue colour for desired OD).*
9.	Pipette **50 µl** of **stopping solution** into each well at the same timed intervals as in step 7.
10.	Read the plate on a microwell plate reader at **450 nm** within 20 minutes after addition of the stopping solution.
■	*If the OD exceeds the upper limit of detection or if a 450nm filter is unavailable, a 405 or 415nm filter may be substituted. The optical densities will be lower, however, this will not affect the results of patient/control samples.*

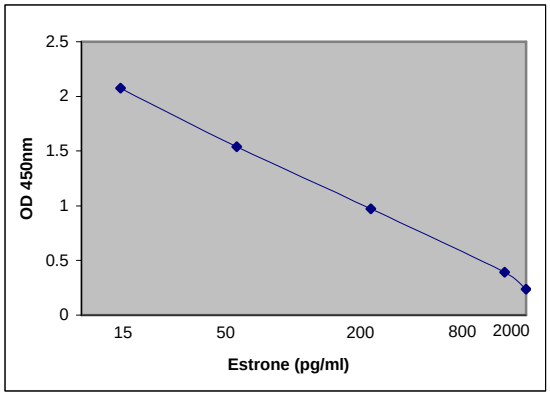
CALCULATIONS

- Calculate the mean optical density of each calibrator duplicate.
- Draw a calibrator curve on semi-log paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis. If immunoassay software is being used, a 4-parameter or 5-parameter curve is recommended.
- Calculate the mean optical density of each unknown duplicate.
- Read the values of the unknowns directly off the calibrator curve.
- If a sample reads more than 2000 pg/ml then dilute it with calibrator A at a dilution of no more than 1:8. The result obtained should be multiplied by the dilution factor.

TYPICAL TABULATED DATA:

Calibrator	OD 1	OD 2	Mean OD	Value (pg/ml)
A	2.162	2.175	2.169	0
B	1.805	1.935	1.870	15
C	1.470	1.473	1.472	50
D	0.697	0.671	0.684	200
E	0.352	0.345	0.349	800
F	0.222	0.213	0.218	2000
Unknown	1.290	1.350	1.320	65

TYPICAL CALIBRATOR CURVE
Sample curve only. **Do not** use to calculate results.



PERFORMANCE CHARACTERISTICS

SENSITIVITY
The lower detection limit is calculated from the standard curve by determining the resulting concentration of the mean OD of Calibrator A (based on 10 replicate analyses) minus 2 SD. Therefore, the sensitivity of the Direct Estrone ELISA kit is **10.0 pg/ml.**

SPECIFICITY (CROSS REACTIVITY)
The following compounds were tested for cross-reactivity with the Direct Estrone ELISA kit with estrone cross-reacting at 100%.

Steroid	%Cross Reactivity
Estrone	100
Estrone-3-Sulfate	4.9
17β-Estradiol	2.2
Estrone-3-Glucuronide	1.2
17ß-Estradiol-3-Glucuronide	0.14

The following steroids were tested but cross-reacted at less than 0.1%: Androstenedione, Cholesterol, Corticosterone, Cortisol, Cortisone, DHEAS, Diethylstilbesterone, Estriol, 17ß-Estradiol-3-Glucuronide, Estradiol-Sulfate, Progesterone, 17-OH Progesterone and Testosterone.

INTRA-ASSAY PRECISION
Three samples were assayed ten times each on the same calibrator curve. The results (in pg/ml) are tabulated below:

Sample	Mean	SD	CV%
1	55.40	4.50	9.1
2	250.50	16.78	6.7
3	1563.57	112.78	7.2

INTER-ASSAY PRECISION
Three samples were assayed ten times over a period of four weeks. The results (in pg/ml) are tabulated below:

Sample	Mean	SD	CV%
1	55.62	6.53	11.7
2	260.12	17.96	6.9
3	1478.63	145.63	9.9

RECOVERY
Spiked samples were prepared by adding defined amounts of estrone to three patient serum samples. The results (in pg/ml) are tabulated below:

Sample	Obs.Result	Exp.Result	Recovery%
1	52	-	-
Unspiked	315	252	125
+200	557	452	120
+400	1235	1052	117
+1000			
2	75	-	-
Unspiked	493	450	110
+375	505.23	559.81	90.3
+750	712.44	794.88	89.6
+1500			
3	720.11	-	-
Unspiked	758.13	837.64	90.5
+200	856.46	955.17	89.7
+400	1013.61	1190.24	85.1
+1000			

LINEARITY
Three patient serum samples were diluted with calibrator A. The results (in pg/ml) are tabulated below:

Sample	Obs.Result	Exp.Result	Recovery%
1	340.67	-	-
1:2	165.35	170.34	97.1
1:4	95.39	85.17	112.0
1:8	48.47	42.58	113.8
2	1086.01	-	-
1:2	508.58	543.00	93.7
1:4	232.11	271.50	85.5
1:8	114.95	135.75	84.7
3	1313.21	-	-
1:2	612.98	656.61	93.4
1:4	318.63	328.30	97.1
1:8	134.98	164.15	82.2

COMPARATIVE STUDIES
The Direct Estrone ELISA kit (y) was compared with a competitors coated tube RIA kit (x). The comparison of 29 serum samples yielded the following linear regression results:

$$y=1.134x-7.014$$
$$r^2=0.99$$

EXPECTED NORMAL VALUES
As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

Group	Range (pg/ml)
Males	25-150
Females	25-350
Pregnancy	100-8000

REFERENCES

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Symbols:

+2 +8 °C	Storage temperature		Manufacturer	Σ	Contains sufficient for <n> tests
	Expiry date	LOT	Batch code	I V D	For in-vitro diagnostic use only!
	Consult instructions for use	CONT	Content	CE	CE labelled
	Caution	REF	Catalogue number	RUO	For research use only!