Manufactured For:
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Total Estrogens ELISA

REF IB59134	Rx ONLY	IVD
Effective Date: September 16, 2024	Version: USA-8.0	

1. INTENDED PURPOSE & USE

For the quantitative measurement of Total Estrogens in human serum by an ELISA (Enzyme-Linked Immunosorbent Assay).

This kit is intended for professional use only and is for laboratory use only. For *in vitro* diagnostic use only. Intended to be used manually but may be adaptable to open automated analyzers. The user is responsible for validating the performance of this kit with any automated analyzers.

2. LIMITATIONS RELATED TO INTENDED PURPOSE & USE

1. This test is not intended to be used for screening purposes.
2. This test is not intended for home testing or self-testing.
3. The kit is calibrated for the determination of total estrogens in human serum. The kit is not calibrated for the determination of total estrogens in other specimens of human or animal origin.
4. The results obtained with this kit shall never be used as the sole basis for a clinical diagnosis and for therapeutic decisions.
5. Although common interfering substances have been evaluated with this test, other substances that have not been evaluated such as drugs and the occurrence of heterophilic antibodies in individuals regularly exposed to animals or animal products have the potential of causing interferences.

3. SUPPLEMENTAL INFORMATION

Total estrogens comprise the total quantity of estrone, estradiol, and estriol. 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 to prepare it for the fertilized ovum.

During the follicular phase of the menstrual cycle, the total estrogens level shows a slight increase. The production of total estrogens 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. If fertilization does not occur, the production of total estrogens decreases.

In post-menopausal women, the concentration of all estrogens decreases substantially and estrone becomes the predominant estrogen. In pregnant women, the concentration of all estrogens escalates and estriol becomes the predominant estrogen.

A total estrogens test is commonly indicated to:

- Aid in diagnosis of sex steroid metabolism related conditions, for example, premature or delayed puberty, and aromatase and 17 alpha-hydroxylase deficiencies.
- Follow-up female hormone replacement therapy in post-menopausal women.
- Prognose antiestrogen therapy, for example, aromatase inhibitor therapy.

4. PRINCIPLE OF THE TEST

The Total Estrogens ELISA is a competitive immunoassay. Competition occurs between total estrogens (estrone, estradiol, and estriol) present in calibrators, controls, specimen samples and an enzyme-labelled antigen (HRP conjugate) for a limited number of anti-estrogen antibody binding sites on the microplate wells. After a washing step that removes unbound materials, the TMB substrate (enzyme substrate) is added which reacts with HRP to form a blue-coloured product that is inversely proportional to the amount of total estrogens present. Following an incubation, the enzymatic reaction is terminated by the addition of the stopping solution, converting the colour from blue to yellow. The absorbance is measured on a microplate reader at 450 nm. A set of calibrators is used to plot a calibrator curve from which the amount of total estrogens in specimen samples and controls can be directly read.

5. PROCEDURAL CAUTIONS AND WARNINGS

1. This kit is for use by trained laboratory personnel (professional use only). For laboratory *in vitro* use only.
2. Practice good laboratory practices when handling kit reagents and specimens. This includes:
   - Do not pipette by mouth.
   - Do not smoke, drink, or eat in areas where specimens or kit reagents are handled.
   - Wear protective clothing and disposable gloves.
   - Wash hands thoroughly after performing the test.
   - Avoid contact with eyes; use safety glasses; in case of contact with eyes, flush eyes with water immediately and contact a doctor.
3. 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.
4. Do not use the kit beyond the expiry date stated on the label.
5. If the kit reagents are visibly damaged, do not use the test kit.
6. Do not use kit components from different kit lots within a test and do not use any component beyond the expiration date printed on the label.
7. All kit reagents and specimens must be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of specimens.
8. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
9. Immediately after use, each individual component of the kit must be returned to the recommended storage temperature stated on the label.
10. A calibrator curve must be established for every run.
11. It is recommended to all customers to prepare their own control materials or serum pools which should be included in every run at a high and low level for assessing the reliability of results.
12. The controls (included in kit) must be included in every run and their results must fall within the ranges stated in the quality control certificate; a failed control result might indicate improper procedural techniques or pipetting, incomplete washing, or improper reagent storage.
13. When dispensing the substrate and stopping solutions, do not use pipettes in which these liquids will come into contact with any metal parts.
14. The TMB Substrate 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.
15. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
16. Samples or controls containing azide or thimerosal are not compatible with this kit, they may lead to false results.
17. Samples values above the measuring range of the kit may be reported as >2500 pg/mL. If further dilution and retesting is required, only calibrator A may be used to dilute serum samples. The use of any other reagent may lead to false results.
18. Avoid microbial contamination of reagents.
19. To prevent the contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, calibrator, and control.
20. To prevent the contamination of reagents, do not pour reagents back into the original containers.
21. Kit reagents must be regarded as hazardous waste and disposed of according to local and/or national regulations.
22. Consumables used with the kit that are potentially biohazardous (e.g., pipette tips, bottles or containers containing human materials) must be handled according to biosafety practices to minimize the risk of infection and disposed of according to local and/or national regulations relating to biohazardous waste.
23. This kit contains 1 M sulfuric acid in the stopping solution component. Do not combine acid with waste material containing sodium azide or sodium hypochlorite.
24. The use of safety glasses, and disposable plastic, is strongly recommended when manipulating biohazardous or bio-contaminated solutions.
25. Proper calibration of the equipment used with the test, such as the pipettes and absorbance microplate reader, is required.
26. If a microplate shaker is required for the assay procedure, the type and speed of shaker required is stated in the REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED section. Both the type and speed of shaker used can influence the optical densities and test results. If a different type of shaker and/or speed is used, the user is responsible for validating the performance of the kit.
27. Do not reuse the microplate wells, they are for SINGLE USE only.
28. To avoid condensation within the microplate wells in humid environments, do not open the pouch containing the microplate until it has reached room temperature.
29. When reading the microplate, the presence of bubbles in the wells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.

6. SAFETY CAUTIONS AND WARNINGS

6.1 BIOHAZARDS

The reagents should be considered a potential biohazard and handled with the same precautions applied to blood specimens. All human specimens should be considered a potential biohazard and handled as if capable of transmitting infections and in accordance with good laboratory practices.

6.2 CHEMICAL HAZARDS

Avoid direct contact with any of the kit reagents. Specifically avoid contact with the TMB Substrate (contains tetramethylbenzidine) and Stopping Solution (contains sulfuric acid). If contacted with any of these reagents, wash with plenty of water and refer to SDS for additional information.

7. SPECIMEN COLLECTION, STORAGE AND PRE-TREATMENT

7.1 Specimen Collection & Storage

Approximately 0.15 mL of serum is required per duplicate determination. Collect 4–5 mL of venous blood into an appropriately labelled tube and allow it to clot. Centrifuge at room temperature and carefully transfer the serum into a new storage tube or container. Serum samples may be stored at 2-8°C for up to 24 hours or at -10°C or lower if the analyses are to be done later.
Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

7.2 Specimen Pre-Treatment

Specimen pre-treatment is not required.

8. REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

1. Calibrated single-channel pipette to dispense 50 µL.
2. Calibrated multi-channel pipettes to dispense 50 µL and 150 µL.
3. Calibrated multi-channel pipettes to dispense 350 µL (if washing manually).
4. Automatic microplate washer (recommended).
5. Microplate shaker:
   a. Orbital shaker (3 mm diameter) set to 600 rpm or
   b. Reciprocating shaker (1.5” stroke length) set to 180 oscillations/minute.
6. Disposable pipette tips.
7. Distilled or deionized water.
8. Calibrated absorbance microplate reader with a 450 nm filter and an upper OD limit of 3.0 or greater.

9. REAGENTS PROVIDED

1. MPL — Microplate

Contents:	One anti-estrogens polyclonal antibody-coated 96-well (12x8) microplate in a resealable pouch with desiccant.
Format:	Ready to Use
Storage:	2–8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for three weeks.

2. HRP CONJ — HRP Conjugate

Contents:	One bottle containing Estrogen-Horse Radish Peroxidase (HRP) conjugate in a protein-based buffer with a non-mercury preservative.
Format:	Ready to Use
Volume:	20 mL/bottle
Storage:	2–8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for three weeks.

3. CAL A – H — Calibrator A – H

Contents:	Eight bottles of calibrator containing specified estrogen concentrations. Protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of an estrogen. Listed below are approximate concentrations, please refer to vial labels for exact concentrations. Concentrations: 0, 25, 50, 100, 250, 500, 1000, 2500 pg/mL.
Format:	Ready to Use
Volume:	Calibrator A: 2.0 mL/bottle Calibrator B-H: 1.0 mL/bottle
Storage:	2–8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for three weeks.

4. CONTROL 1 – 2 — Control 1 – 2

Contents:	Two bottles of control containing different estrogen concentrations. Protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of an estrogen. Refer to the QC certificate for the target values and acceptable ranges.
Format:	Ready to Use
Volume:	1.0 mL/bottle
Storage:	2–8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for three weeks.

5. TMB SUB — TMB Substrate

Contents:	One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.
Format:	Ready to Use
Volume:	16 mL/bottle
Storage:	2–8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for three weeks.

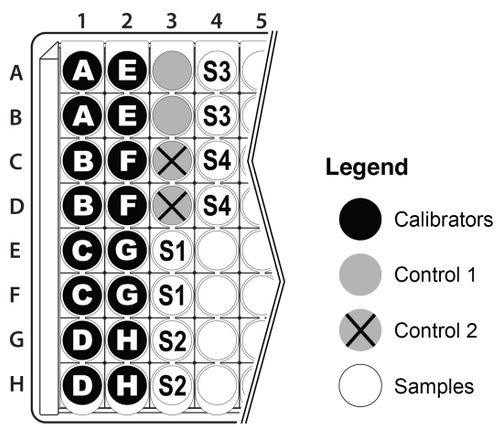
6. STOP Stopping Solution

Contents:	One bottle containing 1M sulfuric acid.
Format:	Ready to Use
Volume:	6 mL/bottle
Storage:	2–8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for three weeks.
Safety:	Refer to product SDS.  Warning

7. WASH BUFF CONC Wash Buffer Concentrate

Contents:	One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.
Format:	Concentrated; Requires Preparation
Volume:	50 mL/bottle
Storage:	2–8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for three weeks. Following Preparation: The wash buffer working solution is stable for 2 weeks following preparation, assuming Good Laboratory Practices are adhered to. To prevent microbial growth, prepare the wash buffer working solution in a clean container and store under refrigerated conditions (2-8°C) when not in use.
Preparation of Wash Buffer Working Solution:	**X10 Dilute 1:10 Before Use** Dilute 1:10 in distilled or deionized water before use. If the whole microplate is to be used dilute 50 mL of the wash buffer concentrate in 450 mL of distilled or deionized water.

10. RECOMMENDED ASSAY LAYOUT



11. ASSAY PROCEDURE

Specimen Pre-Treatment: None

All kit components, controls and specimen samples must reach room temperature prior to 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. After all kit components have reached room temperature, **mix** gently by inversion.
2. **Prepare** the Wash Buffer Working Solution (See section *9. Reagents Provided, 7. Wash Buffer Concentrate*).
3. **Plan** the microplate wells to be used for calibrators, controls, and samples. See section *10. Recommended Assay Layout.* Remove the strips from the microplate frame that will not be used and place them in the bag with desiccant. Reseal the bag with the unused strips and return it to the refrigerator.
4. **Pipette 50 µL** of each calibrator, control, and specimen sample into assigned wells.
5. **Incubate** the microplate on a microplate shaker** for **30 minutes** at room temperature.
6. **Pipette 150 µL** of the HRP Conjugate into each well (the use of a multi-channel pipette is recommended).
7. **Incubate** the microplate on a microplate shaker** for **120 minutes** at room temperature.
8. **Wash** the microplate wells with an automatic microplate washer (preferred) or manually as stated below.

   Automatic: Using an automatic microplate washer, perform a **3-cycle** wash using **350 µL/well** of Wash Buffer Working Solution (3 x 350 µL). One cycle consists of aspirating all wells then filling each well with 350 µL of Wash Buffer Working Solution. After the final wash cycle, aspirate all wells and then tap the microplate firmly against absorbent paper to remove any residual liquid.

   Manually: For manual washing, perform a **3-cycle** wash using **350 µL/well** of Wash Buffer Working Solution (3 x 350 µL). One cycle consists of aspirating all wells by briskly emptying the contents of the wells over a waste container, then pipetting 350 µL of Wash Buffer Working Solution into each well using a multi-channel pipette. After the final wash cycle, aspirate all wells by briskly emptying the contents over a waste container and then tap the microplate firmly against absorbent paper to remove any residual liquid.
9. **Pipette 150 µL** of TMB Substrate into each well (the use of a multi-channel pipette is recommended).
10. **Incubate** the microplate on a microplate shaker** for **30 minutes** at room temperature.
11. **Pipette 50 µL** of Stopping Solution into each well (the use of a multi-channel pipette is recommended) in the same order and speed as was used for addition of the TMB Substrate. Gently tap the microplate frame to mix the contents of the wells.
12. **Measure** the optical density (absorbance) in the microplate wells using an absorbance microplate reader set to 450 nm, within 20 minutes after addition of the Stopping Solution.

** See section *8. Reagents And Equipment Needed But Not Provided* for microplate shaker options

12. CALCULATIONS

1. Calculate the mean optical density for each calibrator, control and specimen sample duplicate.
2. Use a 4-parameter or 5-parameter curve fit with immunoassay software to generate a calibrator curve.
3. The immunoassay software will calculate the concentrations of the controls and specimen samples using the mean optical density values and the calibrator curve.
4. If a sample reads more than 2500 pg/mL and needs to be diluted and retested, then dilute with calibrator A not more than 1:10. The result obtained must be multiplied by the dilution factor.

13. QUALITY CONTROL

When assessing the validity of the test results, the following criteria should be evaluated:

1. The calibrator A mean optical density meets the acceptable range as stated in the QC Certificate.
2. The calibrator with the highest concentration meets the % binding acceptable range as stated in the QC Certificate. % Binding = (OD of calibrator/OD of calibrator A) x 100.
3. The values obtained for the kit controls are within the acceptable ranges as stated in the QC certificate.
4. The results of any external controls that were used meet the acceptable ranges.

14. TYPICAL DATA

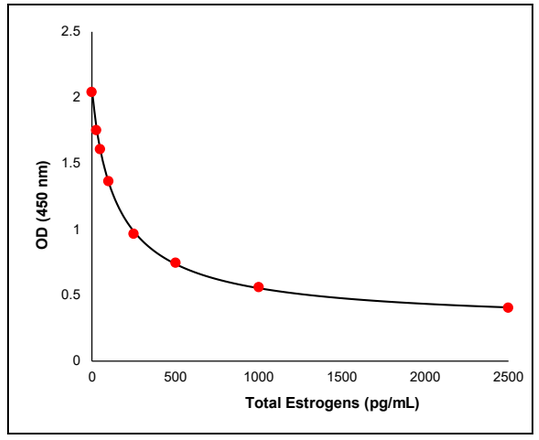
14.1 TYPICAL TABULATED DATA

Sample data only. **Do not** use to calculate results.

Calibrator	Mean OD (450 nm)	% Binding	Value (pg/mL)
A	2.044	100	0
B	1.755	86	25
C	1.609	79	50
D	1.368	67	100
E	0.964	47	250
F	0.744	36	500
G	0.561	27	1000
H	0.407	20	2500
Unknown	0.791	-	400

14.2 TYPICAL CALIBRATOR CURVE

Sample curve only. **Do not** use to calculate results.



15. PERFORMANCE CHARACTERISTICS

15.1 SENSITIVITY

The lower detection limit was calculated following EP17-A2. Sixty replicates of the matrix and a low concentration sample were run in independent tests with two lots of the kit. The Limit of Background was determined to be 5.4 pg/mL and the Limit of Detection was determined to be 12.4 pg/mL.

15.2 SPECIFICITY (CROSS-REACTIVITY)

The following compounds were tested for cross-reactivity in relation to estrogens cross-reacting at 100%.

Compound	% Cross-Reactivity
Estrone	100
17β-Estradiol	100
Estriol	100
11-Deoxycorticosterone	0.4
17-Hydroxyprogesterone	0.3
17α-Estradiol	5.3
Aldosterone	0.2
Androstenedione	0.2
Androsterone	0.2
Cholesterol	0
Corticosterone	< 0.01
Cortisol	< 0.1
DHEA	0.3
DHEAS	0.004
DHT	0.5
Equilin	6.3
Estradiol sulfate	0.1
Estrone sulfate	0.07
Prednisone	0
Pregnenolone	< 0.1
Pregnenolone sulfate	< 0.1
Progesterone	< 0.1
Testosterone	0.3

15.3 INTERFERENCES

Hemoglobin up to 2 g/L, Bilirubin conjugated and unconjugated up to 10 mg/dL, Triglycerides up to 5 mg/mL, Biotin up to 2.4 µg/mL, HAMAS up to 1.2 µg/mL, and Rheumatoid Factor up to 1500 IU/mL did not interfere with the assay.

Note on Fulvestran

Estradiol immunoassays have been reported to show interference from the drug Fulvestran (Faslodex®). This cross-reactivity can cause falsely elevated estrogen levels in patients under Fulvestrant treatment.

The following results were obtained with the Total Estrogens ELISA kit after pooled serum samples from three cohorts were spiked to a concentration of 25 ng/mL of Fulvestran.

Sample	Unspiked Sample (pg/mL)	Sample Spiked to 25 ng/mL Fulvestran (pg/mL)
Pool 1	106.8	128.6
Pool 2	87.8	105.8
Pool 3	326.4	377.6

The Cmax has been reported as 11.4 ng/mL (Robertson and Harrison, 2004) and 25.1 ng/mL (AstraZeneca Canada, 2017).

References

1. Faslodex® Product Monograph. AstraZeneca Canada, 2017.
2. Robertson JFR and Harrison M. Fulvestran Pharmacokinetics and pharmacology. *British Journal of Cancer.* 2004; 90:S7–S10.

15.4 PRECISION

The experimental protocol used a nested components-of-variance design with 10 testing days, two runs per scientist per day, and two replicate measurements per run (a 10 x 2 x 2 x 2 design) for each sample. The results were analyzed with a two-way nested ANOVA and summarized in the table below.

Sample	Mean (pg/mL)	Within Run SD (pg/mL)	Within Run CV%	Between Run SD (pg/mL)	Between Run CV%	Total SD (pg/mL)	Total CV%
1	104.6	6.6	6.3	8.3	8.0	11.9	11.4
2	56.5	5.3	9.3	7.0	12.4	8.8	15.5
3	377.2	17.6	4.7	10.8	2.9	24.4	6.5
4	83.3	4.7	5.7	4.2	5.0	7.1	8.5
5	100.2	6.0	6.0	7.5	7.4	9.9	9.9
6	251.8	10.3	4.1	13.3	5.3	17.0	6.8
7	365.9	16.8	4.6	52.2	14.3	54.8	15.0
8	1276.7	78.9	6.2	46.8	3.7	98.0	7.7

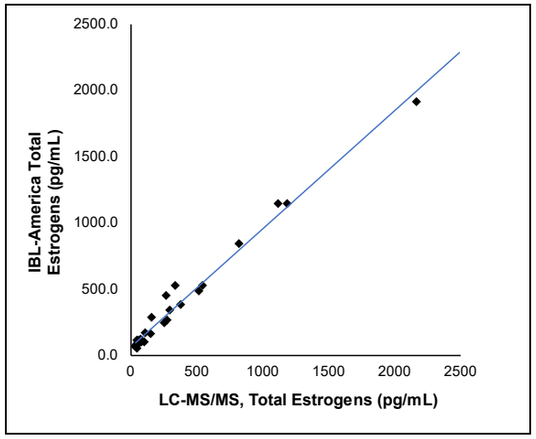
15.5 LINEARITY

The linearity study was performed with four human serum samples covering the range of the assay and following CLSI guideline EP06-A. The samples were diluted in calibrator A at several equidistant concentration levels and up to ten percent (1:10), tested in duplicate, and the results compared to the predicted concentration. The statistical analysis shows that the assay is sufficiently linear up to a 1:10 dilution when using calibrator A as the diluent.

15.6 COMPARATIVE STUDIES

The IBL-America Total Estrogens ELISA kit (y) was compared to Liquid Chromatography-Tandem Mass Spectrometry (x) Estrogens method. The comparison of 27 serum samples yielded the following linear regression results:

y = 0.89x + 62, r = 0.99



16. REFERENCE RANGES

Reference ranges (95%) were established using samples obtained from individuals of diverse races. Each laboratory shall establish their own range of reference values.
Each laboratory shall establish their own reference ranges.

Group	N	Median (pg/mL)	95% Confidence Range (pg/mL)
Pre-menopausal Females, Cycle			
1–10 days	40	120	16 – 328
11–20 days	40	136	34 – 501
21–30 days	40	168	48 – 350
Post-menopausal Females	120	74	40 – 244
Adult Males	120	104	56 – 213

17. SYMBOLS GLOSSARY

Symbol	Definition	Symbol	Definition
REF	Catalogue number		Manufacturer
LOT	Batch code		Date of manufacture
IVD	In vitro diagnostic medical device		Biological risks
UDI	Unique Device Identifier		Consult instructions for use
X #	Dilute 1:# Before Use	Rx ONLY	Prescription only: Device restricted to use by or on the order of a physician
QTY	Quantity		Keep away from sunlight
	Use-by date	EC REP	Authorized representative in the European Community/ European Union
	Do not re-use		Temperature limit
	Caution		Contains sufficient for <n> tests
LYO	Lyophilized	RUO	For Research Use Only. Not for use in diagnostic procedures.

The definitions of symbols used for kit component names are described in the *Reagents Provided* section.

18. CHANGE HISTORY

Previous Version:	USA-7.0	**New Version:**	USA-8.0
Changes:	**9. REAGENTS PROVIDED** **4. Control 1 – 2** **Deletion:** The concentration of the controls was verified by a second party with a CDC HoSt certified method. Build: *v1.3D* BASE*: v8.0*		

19. GENERAL INFORMATION

Manufactured For and Distributed By:

Immuno-Biological Laboratories, Inc.
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Minneapolis, MN 55432, USA
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Email: info@ibl-america.com
Web: www.ibl-america.com

Product Complaints

In the case of product complaints, the user shall submit in writing to the distributor or manufacturer a description of the complaint and provide accompanying data and/or information.

Warranty

IBL-America guarantees that the product is free of defects and will perform within the product specifications when the product is used prior to the expiration date, according to the intended purpose and use, and according to the instructions for use provided with the product. Any deviations from the intended purpose and use, instructions for use, modifications to kit components or use beyond the expiration date will invalidate any warranty claims.

Limitation of Liability

IBL-Americaliability in all circumstances whether in tort (including negligence) or at common law, and for any damage or loss, including but not limited to loss of profit and loss of sales, suffered whether direct, indirect, consequential, incidental or special is limited to the purchase price of the product(s) in question.