Diagnostics Biochem Canada Inc.
Manufacturing Innovative IVD for the World
Since 1973

Free Estriol ELISA

REF CAN-E-640	Rx ONLY	IVD
Effective Date: January 12, 2023	Version: USA-3.0	

1. INTENDED PURPOSE & USE

For the quantitative measurement of Free Estriol (E3) 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 free estriol in human serum. The kit is not calibrated for the determination of free estriol 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

Since the production of estriol in pregnant women depends on a healthy maternal-placental-fetal system, the estriol concentration is a marker of both placental and fetal normal development and metabolism; hence the determination of serum estriol concentration is instrumental for the assessment of fetus health in advanced pregnancy (Berkane et al, 2017). Due to the significant temporal fluctuations in the concentrations of this hormone, multiple tests are recommended to obtain reliable results (Fleck et al, 2018).

4. PRINCIPLE OF THE TEST

The Free Estriol ELISA is a competitive immunoassay. Competition occurs between estriol present in calibrators, controls, specimen samples and an enzyme-labelled antigen (HRP conjugate) for a limited number of anti-estriol 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 estriol. 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 estriol 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 >30 ng/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.

The calibrators and controls provided with the kit contain material(s) of human origin that have been tested by approved methods and found to be negative for the presence of HBsAg, HIV-1 (NAT), HCV (NAT), HCV antibody and antibodies to HIV 1/2. However, no test method can offer complete assurance that any viable pathogens are absent. Therefore, these components should be considered a potential biohazard and handled with the same precautions as applied to any blood specimen, following 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 0.1 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 at a later date.

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 10 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. Disposable pipette tips.
6. Distilled or deionized water.
7. 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-estriol monoclonal 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 four weeks.

2. HRP CONJ — HRP Conjugate

Contents:	One bottle containing Estriol-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 four weeks.

3. CAL A – F — Calibrator A – F

Contents:	Six bottles of calibrator containing specified estriol concentrations. Protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of estriol. Listed below are approximate concentrations, please refer to vial labels for exact concentrations. Concentrations: 0, 0.05, 0.25, 1, 5, 30 ng/mL
Format:	Ready to Use
Volume:	Calibrator A: 2.0 mL/bottle Calibrator B-F: 1.0 mL/bottle
Storage:	2–8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four weeks.

4. CONTROL 1 – 2 — Control 1 – 2

Contents:	Two bottles of control containing different estriol concentrations. Protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of estriol. 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 four 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 four weeks.

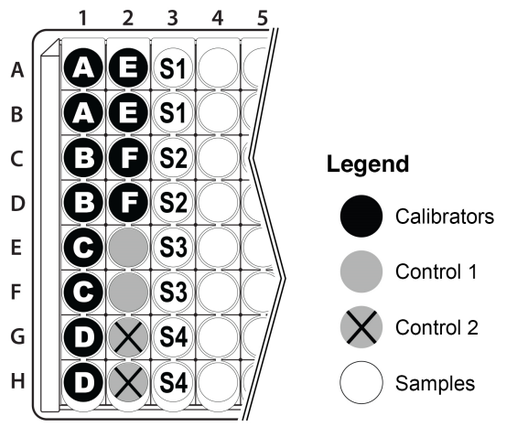
6. STOP — Stopping Solution

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 four 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 four 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 10 µL** of each calibrator, control, and specimen sample into assigned wells.
5. **Pipette 150 µL** of the HRP Conjugate into each well (the use of a multi-channel pipette is recommended).
6. Gently tap the microplate frame for 10 seconds to mix the contents of the wells and **incubate** the microplate at room temperature (no shaking) for **60 minutes**.
7. **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.
8. **Pipette 150 µL** of TMB Substrate into each well (the use of a multi-channel pipette is recommended).
9. **Incubate** the microplate at room temperature (no shaking) for **15-20 minutes**.
10. **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.
11. **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.

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 30 ng/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

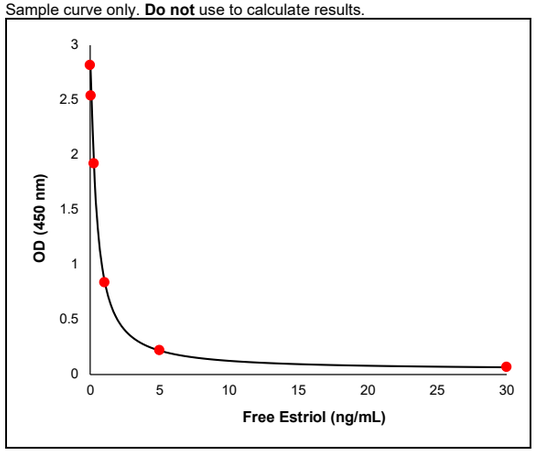
14.1 TYPICAL TABULATED DATA

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

Calibrator	Mean OD (450 nm)	% Binding	Value (ng/mL)
A	2.822	100	0
B	2.542	90	0.05
C	1.924	68	0.25
D	0.840	30	1
E	0.224	8	5
F	0.072	3	30
Unknown	1.929	-	0.24

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-A. 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 0.027 ng/mL and the Limit of Detection was determined to be 0.058 ng/mL.

15.2 SPECIFICITY (CROSS-REACTIVITY)

The following compounds were tested for cross-reactivity with estriol cross-reacting at 100%.

Compound	% Cross-Reactivity
Estriol	100
Estriol-3-Sulfate	0.6
Estriol-3-Glucuronide	1.3
Estradiol	< 0.1
17α-Estradiol	< 0.1
Estradiol Sulfate	< 0.01
Estrone	< 0.1
Estrone Sulfate	< 0.01
Cholesterol	< 0.0001
Corticosterone	< 0.01
DHEAS	< 0.1
Equilin	< 0.1
Prednisone	< 0.001

15.3 INTERFERENCES

Hemoglobin up to 2 g/L, Bilirubin conjugated and unconjugated up to 20 mg/dL, Triglycerides up to 5 mg/mL, Biotin up to 10 µg/mL, Daidzein, Genistein and Resveratrol each up to 200 ng/mL, HAMAS up to 1.2 µg/mL, and Rheumatoid Factor up to 1.2 IU/mL did not interfere with the assay.

15.4 PRECISION

The experimental protocol used a nested components-of-variance design with 10 testing days, two lots and two scientists per day. Each scientist ran two tests with two lots 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 are summarized in the table below.

Sample	Mean (ng/mL)	Within Run SD	Within Run CV%	Total SD	Total CV%
1	0.167	0.023	13.6	0.026	15.6
2	0.264	0.032	12.3	0.036	13.8
3	0.946	0.062	6.5	0.066	7.0
4	4.841	0.326	6.7	0.366	7.6
5	11.89	1.107	9.3	1.148	9.7
6	16.10	1.621	10.1	1.639	10.2
7	3.544	0.232	6.5	0.256	7.2
8	1.927	0.110	5.7	0.119	6.2
9	5.932	0.403	6.8	0.448	7.5
10	9.127	0.606	6.6	0.619	6.8

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-fold (1:10), tested in duplicate, and the results (y) compared to the predicted concentration (x). The statistical analysis shows that the assay is sufficiently linear up to a 1:10 dilution throughout the dynamic range of the kit when using calibrator A as the diluent.

Linear regression results:

y = 1.04x - 0.71; r = 0.99

15.6 COMPARATIVE STUDIES

The DBC Free Estriol ELISA kit (y) was compared to a commercial Estriol Immunofluorescence assay (x) used for IVD. The comparison of 61 human serum samples yielded the following linear regression results:

y = 0.92x - 0.12, r = 0.99

16. REFERENCE RANGES

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

ND = Not detectable; lower than the LoD.

Group	N	Median (ng/mL)	95% Range (ng/mL)	Total Range (ng/mL)
Adult Males and Non-Pregnant Females	120	< 0.058	ND – 0.11	ND – 0.12
Pregnant Females First Trimester	30	0.15	–	ND – 2.95
Pregnant Females Second Trimester	50	1.20	0.46 – 3.04	0.45 – 3.07
Pregnant Females Third Trimester	25	9.5	–	3.6 – 14.3

17. LITERATURE

1. Berkane N, Liere P, Oudinet JP, Hertig A, Lefèvre G, Pluchino N, Schumacher M, Chabbert-Buffet N. From Pregnancy to Preeclampsia: A Key Role for Estrogens. *Endocrine Reviews*. 2017;38:123–144.
2. Fleck SC, Twaddle NC, Churchwell MI, Doerge DR, Pande P, Teeguarden JG. Comparative estrogenicity of endogenous, environmental and dietary estrogens in pregnant women I: Serum levels, variability and the basis for urinary biomonitoring of serum estrogenicity. *Food and Chemical Toxicology*. 2018;115:511–522.
3. Kos-Kudła B, et al. Comparative studies of serum and salivary estriol concentrations in third trimester of normal pregnancy. *Med Sci Monit*.1999;5:285–288.
4. Falah N, et al. Estriol review: Clinical applications and potential biomedical importance. Open Access Text. *Clin Res Trials*. 2015;1:29–33. doi: 10.15761/CRT.1000109
5. Johnsson VL, et al. Plasma progesterone, estradiol, and unconjugated estriol concentrations in twin pregnancies: Relation with cervical length and preterm delivery. *Acta Obstet Gynecol Scand*. 2019;98(1):86–94.

18. 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.			

19. CHANGE HISTORY

Previous Version:	2.0 (Combined)	New Version:	USA-3.0
Changes:	New IFU format with numbered headings. **HEADING** Removal of country-specific regulatory information. Addition of Rx ONLY symbol. **1. INTENDED PURPOSE & USE** Addition: 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 and 2 added. **5. PROCEDURAL CAUTIONS AND WARNINGS** Additional cautions and warnings added. Some previous limitations added to this section. **9. REAGENTS PROVIDED** Addition of symbols for all components and safety information if applicable. In-use stability statement added for all components. Control low and high now called control 1 and 2, respectively. **11. ASSAY PROCEDURE** Component names revised to match symbol definitions. **12. CALCULATIONS** Removed instructions for manually plotting calibrator curve. **13. QUALITY CONTROL** New section added. **18. SYMBOLS GLOSSARY** Addition of symbols and definitions. **19. CHANGE HISTORY** New section added. **20. GENERAL INFORMATION** Addition of product complaints, warranty and limitation of liability sections. Build: *v1.3D*		

20. GENERAL INFORMATION

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Tel: (519) 681-8731
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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

DBC 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

DBC liability 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.