

## Glucagon ELISA

**REF** CAN-GLN-390

**RUO**

Effective Date: August 23, 2024

Version: RUO-1.0

### 1. INTENDED PURPOSE & USE

For the quantitative measurement of Glucagon in human EDTA plasma by an ELISA (Enzyme-Linked Immunosorbent Assay).

For Research Use Only. Not for use in diagnostic procedures.

### 2. LIMITATIONS RELATED TO INTENDED PURPOSE & USE

- This kit is intended for research use only and is not to be used for any diagnostic procedures.

### 3. PRINCIPLE OF THE TEST

The Glucagon ELISA is a two-step capture or 'sandwich' type immunoassay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for glucagon is immobilized onto the microplate and another monoclonal antibody specific for a different epitope of glucagon is conjugated to horse radish peroxidase (HRP conjugate). In the first incubation step, glucagon present in the specimen samples, calibrators and controls is bound to the antibody immobilized onto the microplate. Excess and unbound materials are removed by a washing step. In the second incubation step, HRP conjugate antibody (HRP conjugate) is added, which binds specifically to any immobilized glucagon, thus forming a sandwich complex. Unbound HRP conjugate is removed by a washing step. Next, the TMB substrate (enzyme substrate) is added which reacts with HRP to form a blue coloured product that is directly proportional to the amount of glucagon present. 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 glucagon in specimen samples and controls can be directly read.

### 4. PROCEDURAL CAUTIONS AND WARNINGS

- This kit is for use by trained laboratory personnel (professional use only). For laboratory *in vitro* use only.
- 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.
- 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.
- Do not use the kit beyond the expiry date stated on the label.
- If the kit reagents are visibly damaged, do not use the test kit.
- 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.
- 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.

- When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
- When adding deionized water for the reconstitution of lyophilized components, it is recommended to pre-wet the pipette tip to ensure an accurate transfer of water.
- Immediately after use, each individual component of the kit must be returned to the recommended storage temperature stated on the label. For reconstituted lyophilized reagents, follow storage requirements in section 8. *Reagents Provided*.
- A calibrator curve must be established for every run.
- It is recommended to all customers to prepare their own control materials or plasma pools which should be included in every run at a high and low level for assessing the reliability of results.
- 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.
- When dispensing the substrate and stopping solutions, do not use pipettes in which these liquids will come into contact with any metal parts.
- 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.
- Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored plasma.
- Samples or controls containing azide or thimerosal are not compatible with this kit, they may lead to false results.
- Plasma samples with a known glucagon concentration of less than 50 pg/mL may be used to dilute plasma samples with glucagon concentrations higher than 352 pg/mL. Otherwise, results may be reported as "> 352 pg/mL". The use of any other reagent will lead to false results.
- Avoid microbial contamination of reagents.
- To prevent the contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, calibrator, and control.
- To prevent the contamination of reagents, do not pour reagents back into the original containers.
- Kit reagents must be regarded as hazardous waste and disposed of according to local and/or national regulations.
- 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.
- 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.
- The use of safety glasses, and disposable plastic, is strongly recommended when manipulating biohazardous or bio-contaminated solutions.
- Proper calibration of the equipment used with the test, such as the pipettes and absorbance microplate reader, is required.
- 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.
- Do not reuse the microplate wells, they are for SINGLE USE only.
- To avoid condensation within the microplate wells in humid environments, do not open the pouch containing the microplate until it has reached room temperature.
- 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.

## 5. SAFETY CAUTIONS AND WARNINGS

### 5.1 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 calibrator stock and controls provided with the kit contain a material of human origin that has been found to be not infectious. 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.

### 5.2 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.

## 6. SPECIMEN COLLECTION, STORAGE AND PRE-TREATMENT

### 6.1 Specimen Collection & Storage



**Follow the specimen collection procedure steps in the order provided below to avoid any delays that could potentially affect the stability of specimen samples. K2 and K3 EDTA collection tubes are suitable for plasma sample collection.**

Approximately 0.3 mL of K2 or K3 EDTA plasma is required per duplicate determination.

- Prior to sample collection, place a K2 or K3 EDTA plasma collection tube into a container of ice for at least 10 minutes.
- Collect 4–5 mL of venous blood into an appropriately labelled pre-cooled EDTA plasma collection tube.
- Mix the tube by inverting several times.
- Place the collection tube into a container of ice to keep cool prior to centrifugation.
- Centrifuge the tube at 2000x g for 10 minutes.
- Immediately** transfer the plasma into a labelled tube and store at ≤ -20°C. It is important to complete this step promptly to avoid sample degradation.
- Plasma samples must be stored frozen at ≤ -20°C and are stable for up to 3 months. Avoid no more than 2 freeze/thaw cycles.

### 6.2 Specimen Pre-Treatment

Specimen pre-treatment is not required.

## 7. REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

- Calibrated single-channel pipette to dispense 40 – 120 µL and 500 – 1000 µL.
- Calibrated multi-channel pipettes to dispense 50 µL and 100 µL.
- Calibrated multi-channel pipettes to dispense 350 µL (if washing manually).
- Automatic microplate washer (recommended).
- Disposable pipette tips.
- Distilled or deionized water.
- Calibrated absorbance microplate reader with a 450 nm filter and an upper OD limit of 3.0 or greater.
- Polypropylene or HDPE tubes for calibrator preparation (e.g., polypropylene microcentrifuge tubes).
- Centrifuge capable of 2000x g.
- Container with ice.
- Vortex mixer.

## 8. REAGENTS PROVIDED

### 1. **MPL** Microplate

Contents:	One anti-glucagon 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 3 months.

### 2. **HRP CONJ CONC** HRP Conjugate Concentrate

Contents:	One bottle containing anti-glucagon monoclonal antibody-Horse Radish Peroxidase (HRP) conjugate in a stabilized buffer with a non-mercury preservative.
Format:	Concentrated; Requires Preparation
Volume:	0.3 mL/bottle
Storage:	2–8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for 3 months. Following Preparation: The HRP conjugate working solution is stable for 8 hours at room temperature following preparation.

### **X101** Dilute 1:101 Before Use

Preparation of HRP Conjugate Working Solution:	Dilute 1:101 in Conjugate Diluent before use (e.g., 40 µL of HRP conjugate concentrate in 4 mL of conjugate diluent). If the whole plate is to be used dilute 120 µL of HRP conjugate concentrate in 12 mL of conjugate diluent.
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### 3. **CAL STK LYO** Calibrator Stock Lyophilized

Contents:	One bottle of calibrator stock containing glucagon in a protein-based buffer with a non-mercury preservative. Used for the preparation of glucagon calibrators.
Format:	Lyophilized and Concentrated; Requires Preparation
Storage:	2–8°C (unopened)
Stability:	Unopened: Stable until the expiry date printed on the label.  After Opening and Reconstitution: Store remaining reconstituted Calibrator Stock at ≤ -20°C for up to 2 months with no more than 2 freeze/thaw cycles.

Reconstitution: Reconstitute the lyophilized Calibrator Stock by adding 0.5 mL of distilled or deionized water to the bottle. Replace the stopper and let stand at room temperature for 2 minutes. Mix gently without foaming before use.



**Only reconstitute the Calibrator Stock immediately prior to the preparation of Glucagon Calibrators.**


Preparation of Calibrators:	See section 9. <i>Preparation of Glucagon Calibrators</i> .
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4.	<b>CONTROL</b>	<b>1 – 2</b>	<b>LYO</b>	<b>Control 1 – 2 Lyophilized</b>
Contents:	Two bottles of lyophilized control containing different glucagon concentrations. Protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of glucagon. Refer to the QC certificate for the target values and acceptable ranges.			
Format:	Lyophilized; Requires Preparation			
Storage:	2–8°C (unopened)			
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening and Reconstitution: Stable for <b>2 hours</b> at room temperature.			
	For long-term storage, store at ≤ -20°C for up to 2 months with no more than 2 freeze/thaw cycles.			
Reconstitution:	Reconstitute each bottle of control (Control 1 & Control 2) by adding 0.5 mL of distilled or deionized water to the bottle. Replace the stopper and let stand at room temperature for 2 minutes. Mix gently without foaming.			

5.	<b>CONJ</b>	<b>DIL</b>	<b>Conjugate Diluent</b>
Contents:	One bottle containing a protein-based buffer with a non-mercury preservative. Used for the preparation of the HRP Conjugate Working Solution.		
Format:	Ready to Use		
Volume:	15 mL/bottle		
Storage:	2–8°C		
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for 3 months.		

6.	<b>CAL</b>	<b>DIL</b>	<b>Calibrator Diluent</b>
Contents:	One bottle containing a protein-based buffer with a non-mercury preservative. Used for the preparation of the Glucagon Calibrators.		
Format:	Ready to Use		
Volume:	15 mL/bottle		
Storage:	2–8°C		
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for 3 months.		

7.	<b>TMB</b>	<b>SUB</b>	<b>TMB Substrate</b>
Contents:	One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.		
Format:	Ready to Use		
Volume:	18 mL/bottle		
Storage:	2–8°C		
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for 3 months.		


8.	<b>STOP</b>	<b>Stopping Solution</b>
Contents:	One bottle containing 1M sulfuric acid.	
Format:	Ready to Use	
Volume:	8 mL/bottle	
Storage:	2–8°C	
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for 3 months.	
Safety:	Refer to product SDS.	
	 <b>Warning</b>	

9.	<b>WASH</b>	<b>BUFF</b>	<b>CONC</b>	<b>Wash Buffer Concentrate</b>		
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 3 months. 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:	<table border="1" style="display: inline-table; vertical-align: middle;"> <tr> <td><b>X10</b></td> <td><b>Dilute 1:10 Before Use</b></td> </tr> </table> 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.				<b>X10</b>	<b>Dilute 1:10 Before Use</b>
<b>X10</b>	<b>Dilute 1:10 Before Use</b>					


## 9. PREPARATION OF GLUCAGON CALIBRATORS

### Materials Required:

- Calibrator Stock.
- Calibrator Diluent.
- Calibrated single-channel pipettes.
- 7 x polypropylene or HDPE tubes. (e.g. polypropylene microcentrifuge tubes).

 **Do not use glass test tubes; Glucagon may bind to glass which can alter the results.**

### Procedure:

 **An accurate preparation of the calibrators is essential to the performance of the test. Please follow good pipetting practices specific to the brand of pipettes being used.**

- Label 7 x polypropylene or HDPE tubes as A, B, C, D, E, F & G, representing calibrators A-G.
- Pipette 960 µL of Calibrator Diluent to tube G.
- Pipette 500 µL of Calibrator Diluent to each tube A - F.
- Reconstitute the Calibrator Stock as stated in section 8. *Reagents Provided, 3. Calibrator Stock Lyophilized.*
- Pipette 40 µL of reconstituted Calibrator Stock to tube G. Vortex the tube to mix thoroughly.
- Immediately store the reconstituted Calibrator Stock at ≤ -20°C for future use.
- Pipette 500 µL from tube G into tube F. Vortex tube F to mix thoroughly.
- Pipette 500 µL from tube F into tube E. Vortex tube E to mix thoroughly.
- Pipette 500 µL from tube E into tube D. Vortex tube D to mix thoroughly.
- Pipette 500 µL from tube D into tube C. Vortex tube C to mix thoroughly.
- Pipette 500 µL from tube C into tube B. Vortex tube B to mix thoroughly.

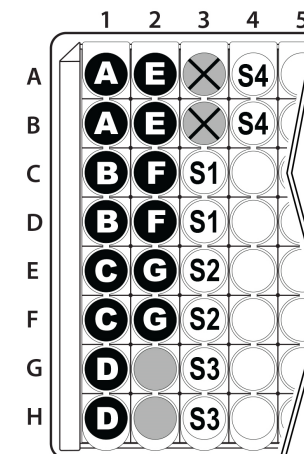


**Use the prepared glucagon calibrators within 2 hours after preparation. Discard any leftover calibrators; do not store for future use.**





### Preparation Summary Table

Calibrator	Glucagon (pg/mL)	Calibrator Diluent	Calibrator
Calibrator Stock Reconstituted	-	-	-
G	352	960 µL	40 µL of Calibrator Stock
F	176	500 µL	500 µL of G
E	88	500 µL	500 µL of F
D	44	500 µL	500 µL of E
C	22	500 µL	500 µL of D
B	11	500 µL	500 µL of C
A	0	500 µL	-


## 10. RECOMMENDED ASSAY LAYOUT



### Legend

-  Calibrators
-  Control 1
-  Control 2
-  Samples

## 11. ASSAY PROCEDURE

	<b>Follow the assay procedure steps in the order provided below to avoid any delays that could potentially affect the stability of components and specimen samples.</b>
All kit components must reach room temperature prior to use. Thaw specimen samples at room temperature. Specimen samples should not be kept at room temperature for more than <b>30 minutes</b> prior to being tested. 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 and specimen samples have reached room temperature, <b>mix</b> gently.
2.	<b>Plan</b> the microplate wells to be used for calibrators, controls, and samples. See section 10. <i>Recommended Assay Layout</i> . 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.
3.	<b>Prepare</b> the HRP Conjugate Working Solution and Wash Buffer Working Solution (See section 8. <i>Reagents Provided</i> , 2. <i>HRP Conjugate Concentrate</i> and 9. <i>Wash Buffer Concentrate</i> ).
4.	<b>Prepare</b> the Glucagon Calibrators and Controls (See section 9. <i>Preparation of Glucagon Calibrators</i> and section 8. <i>Reagents Provided</i> , 3. <i>Calibrator Stock Lyophilized</i> , 4. <i>Control 1-2 Lyophilized</i> ).
5.	<b>Pipette 100 µL</b> of each calibrator, control, and specimen sample into assigned wells.
6.	<b>Incubate</b> the microplate at room temperature (no shaking) for <b>60 minutes</b> . Do not tap the microplate and avoid placing in intense light or air currents. Return specimen samples to frozen storage at ≤ -20°C.
7.	<b>Wash</b> the microplate wells with an automatic microplate washer (preferred) or manually as stated below.  <i>Automatic:</i> Using an automatic microplate washer, perform a <b>3-cycle</b> wash using <b>350 µL/well</b> 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. <i>Manually:</i> Perform a <b>3-cycle</b> wash using <b>350 µL/well</b> 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.	<b>Pipette 100 µL</b> of the HRP Conjugate Working Solution into each well (the use of a multi-channel pipette is recommended).
9.	<b>Incubate</b> the microplate at room temperature (no shaking) for <b>30 minutes</b> . Do not tap the microplate and avoid placing in intense light or air currents.
10.	<b>Wash</b> the microplate wells again as stated in step 7.
11.	<b>Pipette 100 µL</b> of TMB Substrate into each well (the use of a multi-channel pipette is recommended).
12.	<b>Incubate</b> the microplate at room temperature (no shaking) for <b>15 minutes</b> . Do not tap the microplate and avoid placing in intense light or air currents.
13.	<b>Pipette 50 µL</b> 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.
14.	<b>Measure</b> 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

- Calculate the mean optical density for each calibrator, control and specimen sample duplicate.
- Use a 4-parameter or 5-parameter curve fit with immunoassay software to generate a calibrator curve.
- The immunoassay software will calculate the concentrations of the controls and specimen samples using the mean optical density values and the calibrator curve.
- If a sample reads more than 352 pg/mL and needs to be diluted and retested, then dilute it with an EDTA plasma sample that has a glucagon concentration of < 50 pg/mL. Do not dilute the sample more than 1:10. The result obtained must be multiplied by the dilution factor.

### Sample Calculation:

EDTA sample #1 (high sample requiring dilution): >352 pg/mL  
EDTA sample #2 (used to dilute sample #1): 20 pg/mL

EDTA sample #1 was diluted 1:10 using EDTA sample #2.  
40 µL of EDTA sample #1 + 360 µL of EDTA sample #2.

1:10 diluted EDTA sample #1 was tested and had a glucagon concentration of 200 pg/mL.

Calculated concentration of EDTA sample#1:  
= [(Conc. of 1:10 diluted EDTA sample #1) – (Conc. of EDTA sample #2) x % of total volume] x Dilution Factor

= (200 pg/mL – 20 pg/mL x 90%) x 10  
= 182 pg/mL x 10 = 1820 pg/mL

## 13. QUALITY CONTROL

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

- The calibrator A mean optical density meets the acceptable range as stated in the QC Certificate.
- The calibrator with the highest concentration meets the optical density acceptable range as stated in the QC Certificate.
- The values obtained for the kit controls are within the acceptable ranges as stated in the QC certificate.
- The results of any external controls that were used meet the acceptable ranges.

## 14. TYPICAL DATA

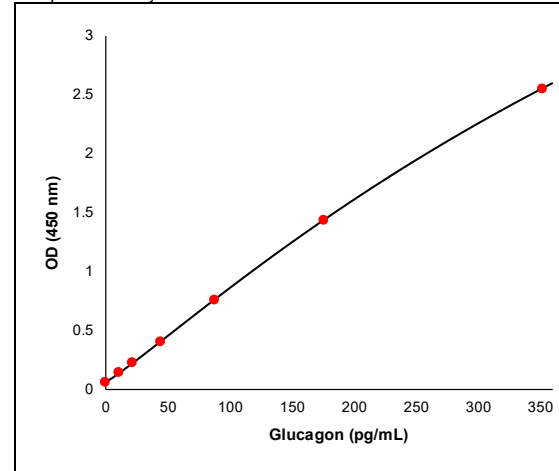
### 14.1 TYPICAL TABULATED DATA

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





















Calibrator	Mean OD (450 nm)	% Binding	Value (pg/mL)
A	0.060	2	0
B	0.141	6	11
C	0.228	9	22
D	0.406	16	44
E	0.758	30	88
F	1.441	56	176
G	2.551	100	352
Unknown	0.430	-	47.1

## 14.2 TYPICAL CALIBRATOR CURVE

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



## 15. SYMBOLS GLOSSARY

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

## 16. CHANGE HISTORY

Previous Version:	-	New Version:	-
Changes:	Build: v1.5D BASE: v1.0		

## 17. GENERAL INFORMATION

	<b>Diagnostics Biochem Canada (DBC) Inc.</b> 384 Neptune Crescent London, Ontario, Canada N6M 1A1 Tel: (519) 681-8731 Fax: (519) 681-8734 e-mail: dbc@dbc-labs.com www.dbc-labs.com
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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.