SALIVARY
Human Total IgM
ELISA KIT

For Research Use Only
Not for use in Diagnostic Procedures

Item No. 1-4002, (Single) 96-Well Kit;
1-4002-5, (5-Pack) 480 Wells

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Intended Use

The Salimetrics® Salivary Human Total IgM ELISA Kit is an enzyme-linked immunoassay specifically designed and validated for the quantitative measurement of human total IgM in oral fluid. It is not intended for diagnostic use. This assay kit was optimized for human salivary research and has not been validated for other human sample types, such as serum or plasma or samples from other species.

Please read the complete kit insert before performing this assay. Failure to follow kit procedure and recommendations for saliva collection and sample handling may result in unreliable values.

For further information about this kit, its application, or the procedures in this insert, please contact the technical service team at Salimetrics or your local sales representative.

Introduction

Immunoglobulin M, or IgM, is one of five classes of antibodies found in humans, and is present as part of both the natural and adaptive immune response (1). Natural IgMs are low affinity pentameric molecules that bind to invading pathogens without requiring prior exposure (2). They act to directly neutralize viruses, activate complement, trigger phagocytosis and drive antibody dependent cell cytotoxicity (3). Adaptive IgM is the first antibody produced in response to invading pathogens and functions in a similar way to natural IgM, but are higher affinity molecules and are an important part of the adaptive immune response (4). IgM is the first immunoglobulin molecule found in the fetus, developing in the second half of pregnancy. The majority of IgM is pentameric and includes a molecule referred to as the J chain, a key structural component also found in polymeric IgA. IgM also forms tetramers and hexamers, however in this case the J chain is absent. In contrast to IgG, IgM antibodies peak early in the course of humoral immune responses waning after several weeks and are thus useful in the determination of recent infections or pathogen exposure. One important use for measuring antigen specific IgM in saliva is during serological surveys when assessing outbreaks of infectious diseases, for instance with the recent COVID-19 outbreak (4). Similar to IgM, the major source of IgM in saliva is blood, and its entry is through gingival crevicular fluid (GCF). Due to this, the total amount of IgM can vary depending on the level of serum component representation. Therefore, when measuring pathogen specific IgM, total IgM can be used to qualify a saliva sample to assure sufficient levels of total IgM in the saliva sample and provide confidence in the pathogen specific IgM results. In this regard, total IgM may be essential to prove a negative pathogen specific test result. In addition, this assay may be used to qualify samples for testing after sample storage.

Test Principle

This is an indirect sandwich ELISA kit. A "sandwich" is formed when the pre-coated capture Anti-Human IgM antibody present on the plate binds IgM in standards & samples, which is then bound by the Anti-Human IgM detection antibody linked to horseradish peroxidase. After each incubation, unbound components are washed away. Bound Anti-Human IgM Antibody Enzyme Conjugate is then added and the levels measured by the reaction of the horseradish peroxidase (HRP) enzyme to the substrate tetramethylbenzidine (TMB). This reaction produces a blue color. A yellow color is formed after stopping the reaction with an acidic solution. The optical density is read on a standard plate reader at 450 nm. The total amount of IgM Antibody Enzyme Conjugate detected is directly proportional to the amount of Total Human IgM present in the sample.

Safety Precautions

Read Safety Data Sheets before handling reagents.

Hazardous Ingredients

Liquid Stop Solution is caustic; use with care. We recommend the procedures listed below for all kit reagents.

Handling

Follow good laboratory safety practices when handling kit reagents. Personal protective equipment is recommended including laboratory coats, gloves, and safety goggles. Wipe up spills using appropriate absorbent materials while wearing protective clothing. Follow local regulations for disposal.

Emergency Exposure Measures

In case of contact, immediately wash skin or flush eyes with water for 15 minutes. Remove contaminated clothing. If inhaled, remove individual to fresh air. If individual experiences difficulty breathing call a physician.

The above information is believed to be accurate but is not all-inclusive. This information should be used only as a guide. Salimetrics will not be liable for accidents or damage resulting from misuse of product.

Safety Data Sheets are available by contacting Salimetrics at support@salimetrics.com (See www.salimetrics.com for alternative contact options).

General Kit Use Advice

- This kit uses break-apart microtiter strips. You may run less than a full plate. Unused wells must be stored at 2-8°C in the foil pouch with desiccant and used in the frame provided.
- Avoid microbial contamination of opened reagents. Salimetrics recommends using opened reagents within one month. Store all reagents at 2-8°C.
- The quantity of reagent provided with a single kit is sufficient for three partial runs. The volume of wash buffer and enzyme conjugate prepared for assays using less than a full plate should be scaled down accordingly, keeping the same dilution ratio.
- Do not mix components from different lots of kits.
- We recommend saving all reagents until data analysis has confirmed a successful run to facilitate troubleshooting if necessary.
- Prior to sample addition, please label each strip to assure plate orientation and sample order when data is acquired on plate reader.
- To ensure highest quality assay results, pipetting of samples and reagents must be done as quickly as possible (without interruption) across the plate. Ideally, the process should be completed within 20 minutes or less.
- When using a multichannel pipette to add reagents, always follow the same sequence when adding all reagents so that the incubation time is the same for all wells.
- When running multiple plates, or multiple sets of strips, a standard curve must be run with each individual plate and/or set of strips.
- The temperature of the laboratory may affect assays. Salimetrics kits have been validated at 68-74°F (20-23.3°C). Higher or lower temperatures may affect OD values.
- Routine calibration of pipettes and other equipment is critical for the best possible assay performance.
- When mixing plates during assay procedures, avoid speeds that spill the contents of the wells.

Storage

All unopened components of this kit are stable at 2-8°C until the kit's expiration date.

Specimen Collection

Acidic or high sugar foods can compromise assay performance by lowering sample pH and influencing bacterial growth. To minimize these factors, rinse mouth thoroughly with water 10 minutes before sample is collected.

Collect whole saliva by unstimulated passive drool. Donors may tilt the head forward, allowing the saliva to pool on the floor of the mouth, and then pass the saliva through the SalivaBio Collection Aid (SCA) into a polypropylene vial. Collection protocols/methods are available online at www.salimetrics.com (https://salimetrics.com/how-do-i-collect-saliva/) or upon request.

Record the time and date of specimen collection.

The major source of IgM is serum derived and may therefore be flow rate dependent. However, in the case where total IgM levels are used to normalize or qualify a sample, flow rate is not relevant. If total IgM levels are of interest in themselves, then the impact of flow rate should be determined. It is therefore advisable to collect data on saliva flow in case correction for flow rate should be necessary, or to allow for future testing of archived samples for additional biomarkers that may be sensitive to flow rate. We recommend you measure the amount of time needed to collect the desired volume of saliva, in order to determine the flow rate (mL/min). The measured concentration should then be multiplied by the flow rate in order to express the result as product measured per unit of time. Protocols for flow-rate conversion are available on request.

Sample Handling and Preparation

After collection, it is important to keep samples cold in order to avoid bacterial growth (and loss of IgM) in the specimen. Refrigerate sample within 30 minutes and freeze at or below -20ºC within 4 hours of collection. Samples may be stored at -20ºC for up to 6 months. For long term storage, refer to the Salimetrics Collection and Handling Advice page on our website.

On day of assay, thaw the saliva samples completely, vortex, and centrifuge at 1500 x g for 15 minutes. Freezing saliva samples will precipitate mucins. Centrifuging removes mucins and other particulate matter which may interfere with antibody binding and affect results. Samples should be at room temperature before making dilutions. Pipette clear sample into appropriate dilution tubes. Re-freeze saliva samples as soon as possible after running assay. Re-centrifuge saliva samples each time that they are thawed. Avoid multiple freeze-thaw cycles. IgM levels are minimally affected by freeze-thaw cycles.

Saliva samples must be diluted for this assay. See Procedure for details.

Materials Supplied with Single Kit

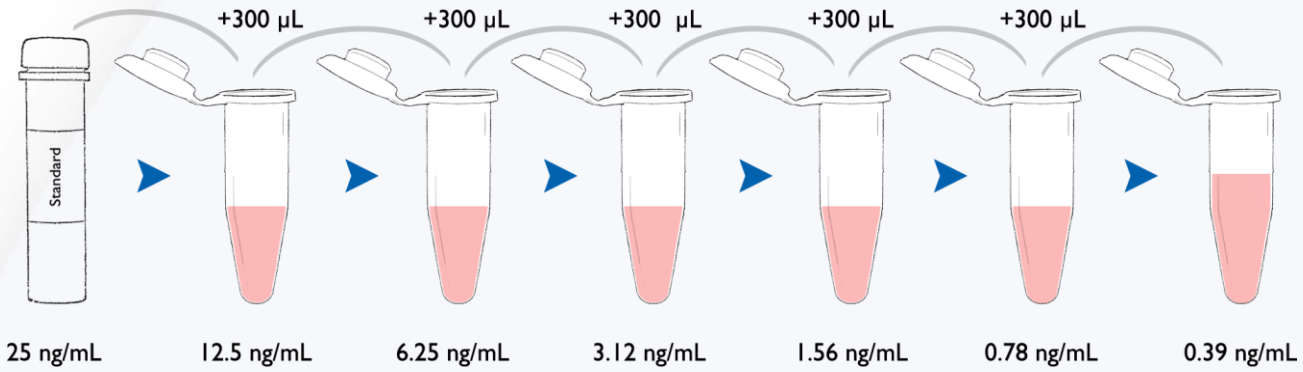
	Item	Quantity/Size
1	**IgM ELISA Microtitre Break-Apart Plate** Coated with Goat Anti-Human IgM antibodies.	1/96 well
2	**IgM Standard** 25 ng/mL formulated for stability when stored at 4ºC. Prepare and serially dilute before use according to Reagent Preparation. Contains: IgM, buffer, preservative.	1 vial / 2 mL
3	**IgM Controls** High and Low. Contain: IgM, buffer, preservative.	2 vials / 1 mL each
4	**IgM Enzyme Conjugate** Concentrate. Dilute before use with IgM Assay Diluent. (See step 7 of Procedure.) Contains: Goat Anti-Human IgM antibody conjugated to HRP, preservative.	1 vial / 100 µL
5	**IgM Assay Diluent** Contains: phosphate buffer, pH indicator, preservative.	1 bottle / 60 mL
6	**Wash Buffer Concentrate 10X** Dilute before use according to Reagent Preparation. Contains: phosphate buffer, detergent, preservative.	1 bottle / 100 mL
7	**TMB Substrate Solution** Non-toxic, ready to use.	1 bottle / 25 mL
8	**Stop Solution**	1 bottle / 12.5 mL
9	**Adhesive Plate Covers**	2

Materials Needed But Not Supplied

- Precision pipettes to deliver 10 µL, 35 µL, 300 µL, and 490 µL
- Precision multichannel pipette to deliver 50 µL and 100 µL
- Vortex
- Plate rotator with 0.08 - 0.17-inch orbit capable of 500 rpm
- Microplate reader with capabilities to read 450 nm and 620 to 630 nm
- Computer software for data reduction
- Reagent reservoirs
- Deionized water
- One disposable polypropylene tube to hold at least 14 mL
- Small disposable polypropylene tubes for dilution of standard & samples
- Pipette tips
- Serological pipette to deliver up to 14 mL
- Centrifuge capable of 1500 x g

Reagent Preparation

- Bring all reagents to room temperature and mix before use. A minimum of 1.5 hours is recommended for the 60 mL of Assay Diluent to come to room temperature.
- Bring Microtiter Plate to room temperature before use. ***It is important to keep the foil pouch with the plate strips closed until warmed to room temperature, as humidity may have an effect on the coated wells.***
- Prepare 1X wash buffer by diluting Wash Buffer Concentrate (10X) 10-fold with room-temperature deionized water (100 mL of Wash Buffer Concentrate (10X) to 900 mL of deionized water). ***Dilute only enough for current day's use and discard any leftover reagent.*** (If precipitate has formed in the concentrated wash buffer, it may be heated to 40°C for 15 minutes. Cool to room temperature before use in assay).
- Prepare serial dilutions of the IgM Standard as follows:
    - Label six polypropylene microcentrifuge tubes or other small tubes 2 through 7.
    - Pipette 300 µL of IgM Assay Diluent into tubes 2 through 7.
    - Serially dilute the standard 2X by adding 300 µL of the 25 ng/mL standard (tube 1) to tube 2. Mix well.
    - After changing pipette tips, remove 300 µL from tube 2 to tube 3. Mix well.
    - Continue for tubes 4, 5, 6 and 7.
    - The final concentrations of standards for tubes 1 through 7 are, respectively, 25 ng/mL, 12.5 ng/mL, 6.25 ng/mL, 3.125 ng/mL, 1.56 ng/mL, 0.78 ng/ml and 0.39 ng/mL.
    - IgM Assay Diluent is used as the Zero Standard.



Procedure

Step 1: Read and prepare reagents according to the Reagent Preparation section before beginning assay. Determine your plate layout. Here is a suggested layout. Standards, controls, and saliva samples should be assayed in duplicate.

	1	2	3	4	5	6	7	8	9	10	11	12
A	25 Std	25 Std	Ctrl-L	Ctrl-L								
B	12.5 Std	12.5 Std	Ctrl-H	Ctrl-H								
C	6.25 Std	6.25 Std	SMP-1	SMP-1								
D	3.125 Std	3.125 Std	SMP-2	SMP-2								
E	1.56 Std	1.56 Std	SMP-3	SMP-3								
F	0.78 Std	0.78 Std	SMP-4	SMP-4								
G	0.39 Std	0.39 Std	SMP-5	SMP-5								
H	0 Std	0 Std	SMP-6	SMP-6								

Step 2: Keep the desired number of strips in the strip holder and place the remaining strips back in the foil pouch. Reseal the foil pouch with unused wells and desiccant. Store at 2-8°C.

Step 3: Pipette 14 mL of IgM Assay Diluent into the disposable tube. Scale down proportionally if using less than the entire plate. Set aside for Step 7.

Step 4:

- Prepare a 1:50 dilution of the saliva by pipetting 10 µL of saliva into 490 µL of IgM Assay Diluent. Mix well.
- Further dilute by pipetting 50 µL of the 1:50 saliva dilution into 450 µL IgM Assay Diluent (1:10). Final dilution is 1:500. Mix Well.

Step 5:

- Pipette 100 µL of IgM Standards, Controls and diluted saliva samples into appropriate wells.
- Pipette 100 µL IgM Assay Diluent into two wells to serve as the Zero Standard.

Place adhesive cover (provided) over plate. Mix plate on a plate rotator at 500 rpm for 5 minutes. Incubate for a total of 1 hour at room temperature.

Step 6: Wash the plate 4 times with 1X wash buffer. A plate washer is recommended. However, washing may be done by gently squirting wash buffer into each well with a squirt bottle, or by pipetting 300 µL of wash buffer into each well and then discarding the liquid over a sink. After each wash, the plate should be thoroughly blotted on paper towels before turning upright. If using a plate washer, blotting is still recommended after the last wash.

Step 7: Dilute the IgM Enzyme Conjugate 1:200 by adding 70 µL of the conjugate to the 14mL tube of IgM Assay Diluent. Scale down proportionally if not using the entire plate. IgM Enzyme Conjugate tube may be centrifuged for a few minutes to bring the liquid down to the tube bottom. Immediately mix the diluted conjugate solution and add 100 µL to each well using a multichannel pipette.

Step 8: Place adhesive cover provided over plate. Mix plate on a plate rotator at 500 rpm for 5 minutes. Incubate for a total of 1 hour at room temperature.

Step 9: Wash the plate 4 times with 1X wash buffer. A plate washer is recommended. However, washing may be done by gently squirting wash buffer into each well with a squirt bottle, or by pipetting 300 µL of wash buffer into each well and then discarding the liquid over a sink. After each wash, the plate should be thoroughly blotted on paper towels before turning upright. If using a plate washer, blotting is still recommended after the last wash.

Step 10: Add 100 µL of TMB Substrate Solution to each well with a multichannel pipette.

Step 11. Incubate the plate in the dark (covered) at room temperature for 30 minutes, mixing for 5 minutes on a plate rotator at 500 rpm.

Step 12: Add 50 µL of Stop Solution with a multichannel pipette.

Step 13:

- Mix on a plate rotator for 3 minutes at 500 rpm. If green color remains, continue mixing until green color turns to yellow. Be sure all wells have turned yellow.

  Caution: Spillage may occur if mixing speed exceeds 600 rpm.
- Wipe off bottom of plate with a water-moistened, lint-free cloth and wipe dry.
- Read in a plate reader at 450 nm. Read plate within 10 minutes of adding Stop Solution. For best results, a secondary filter correction at 620 to 630 nm is recommended.

Quality Control

The Salimetrics High and Low IgM Controls should be run with each assay. The control ranges established at Salimetrics are to be used as a guide. Each laboratory should establish its own range. Variations between laboratories may be caused by differences in techniques and instrumentation.

Calculations

1. Compute the average optical density (OD) for all duplicate wells.
2. Plot the reference standard concentrations on the X axis and the corresponding average optical density on the Y axis.
3. Using the average optical density values of the controls and saliva samples, determine the corresponding concentration of Total Human IgM in ng/mL from the standard curve. We recommend using a non-linear regression curve fit.
4. Multiply the calculated concentrations of the **saliva samples only** by the dilution factor of 500 to obtain final Total Human IgM sample concentrations.

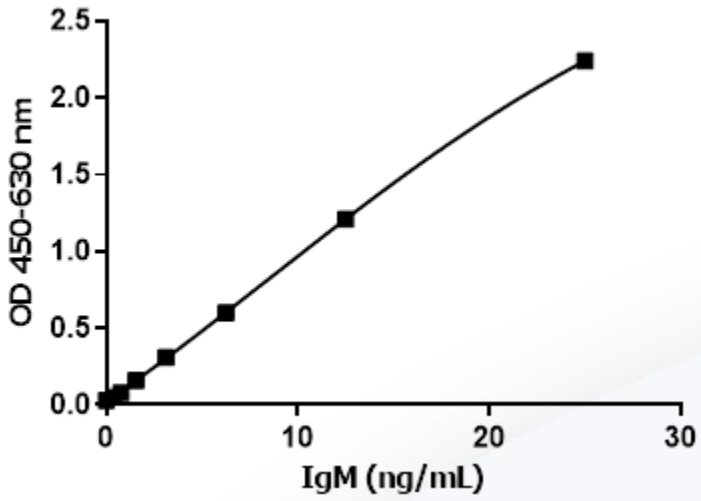
A new Standard Curve must be run with each full or partial plate.

Typical Results

The results shown below are for illustration only and should not be used to calculate results from another assay.

Well	Standard	Average OD	Human IgM (ng/mL)
A1,A2	S1	2.24	25
B1,B2	S2	1.21	12.5
C1,C2	S3	0.6	6.25
D1,D2	S4	0.31	3.125
E1,E2	S5	0.16	1.56
F1,F2	S6	0.08	0.78
G1,G2	S7	0.05	0.39
H1,H2	Zero	0.03	0

Example: Human Total IgM Non-Linear Curve Fit



Limitations

- See "Specimen Collection" recommendations to ensure proper collection of saliva specimens and to avoid interfering substances.
- Any quantitative results indicating abnormal Total IgM levels should be followed by additional testing and evaluation.

Salivary Human Total IgM Performance Characteristics

Precision

The intra-assay precision was determined from the mean of 20 replicates each.

Saliva Sample	N	Mean (µg/mL)	Standard Deviation (µg/mL)	Coefficient of Variation (%)
1	20	3.34	0.06	2%
2	20	2.88	0.05	2%
3	20	2.37	0.06	3%
4	20	1.59	0.05	3%
5	20	1.23	0.02	2%

The inter-assay precision was determined from the mean of average duplicates for 10 separate runs.

Saliva Sample	N	Mean (µg/mL)	Standard Deviation (µg/mL)	Coefficient of Variation (%)
1	20	3.27	0.12	4%
2	20	2.88	0.10	4%
3	20	2.33	0.08	3%
4	20	1.62	0.06	4%
5	20	1.32	0.05	4%

Recovery

Three saliva samples containing different levels of endogenous Human IgM were spiked with known quantities of Human IgM and assayed.

Sample	Endogenous (µg/mL)	Added (µg/mL)	Expected (µg/mL)	Observed (µg/mL)	% Recovery
1	2.30	5.32	7.62	7.40	97%
		2.35	4.65	4.43	95%
		0.27	2.56	2.59	101%
2	0.90	5.32	6.22	6.13	99%
		2.35	3.25	3.11	96%
		0.27	1.17	1.18	101%
3	0.85	2.35	3.20	2.96	92%
		0.27	1.12	1.03	92%

Sensitivity

Analytical Sensitivity

The lower limit of detection (LLOD) was determined by interpolating the mean optical density plus 2 SDs for 10 sets of duplicates at the zero ng/mL standard. The minimal concentration of Total Human IgM that can be distinguished from zero is 0.03 ng/mL.

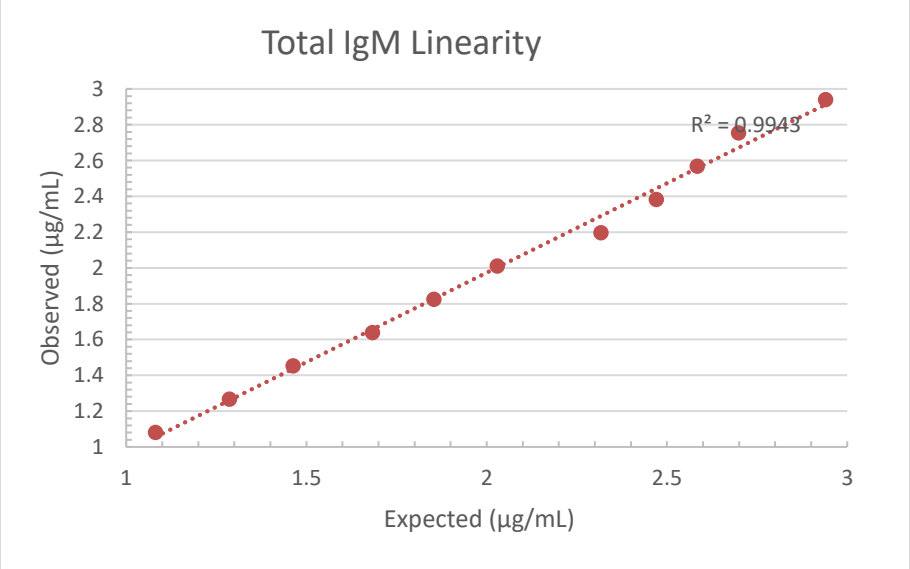
Functional Sensitivity

The functional sensitivity was determined by assaying 60 saliva samples at a concentration level resulting in a CV less than 20%. The functional sensitivity of the salivary IgM ELISA is 0.175 µg/mL.

Linearity of Assay

Two saliva samples were diluted with each other proportionately and assayed.

Sample ID	Percentage of Sample		Rep 1 µg/mL	Rep 2 µg/mL	Mean µg/mL	% CV	Expected	% Recovered
	High (L1)	Low (L11)						
L1 (High)	100%	0%	2.93	2.95	2.94	0%	2.9	100%
L2	90%	10%	2.69	2.71	2.70	1%	2.8	98%
L3	80%	20%	2.61	2.55	2.58	2%	2.6	101%
L4	70%	30%	2.46	2.48	2.47	1%	2.4	104%
L5	60%	40%	2.33	2.31	2.32	1%	2.2	105%
L6	50%	50%	2.06	2.00	2.03	2%	2.0	101%
L7	40%	60%	1.94	1.76	1.85	7%	1.8	102%
L8	30%	70%	1.70	1.67	1.68	1%	1.6	103%
L9	20%	80%	1.52	1.41	1.46	6%	1.5	101%
L10	10%	90%	1.28	1.30	1.29	1%	1.3	102%
L11 (Low)	0%	100%	1.14	1.02	1.08	8%	1.1	100%
							Average =	101%



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Antibody Specificity

Compound	Spiked Concentration (ng/ml)	% Cross-reactivity in total IgM ELISA
IgA	500	ND
IgE	500	ND
IgG	500	ND

ND = None Detected

References

1. Fereidan-Esfahani M, Nayfeh T, Warrington A, Howe CL, Rodriguez M. IgM Natural Autoantibodies in Physiology and the Treatment of Disease. Methods in molecular biology. 2019;1904:53-81.
2. Michaud E, Mastrandrea C, Rochereau N, Paul S. Human Secretory IgM: An Elusive Player in Mucosal Immunity. Trends Immunol. 2020;41(2):141-56.
3. Madar R, Straka S, Baska T. Detection of antibodies in saliva--an effective auxiliary method in surveillance of infectious diseases. Bratisl Lek Listy. 2002;103(1):38-41.
4. Brandtzaeg P. Do salivary antibodies reliably reflect both mucosal and systemic immunity? Annals of the New York Academy of Sciences. 2007;1098:288-311.

Seller's Limited Warranty

"Seller warrants that all goods sold hereunder will be free from defects in material and workmanship. Upon prompt notice by Buyer of any claimed defect, which notice must be sent within thirty (30) days from date such defect is first discovered and within three months from the date of shipment, Seller shall, at its option, either repair or replace the product that is proved to Seller's satisfaction to be defective. All claims should be submitted in written form. This warranty does not cover any damage due to accident, misuse, negligence, or abnormal use. Liability, in all cases, will be limited to the purchased cost of the kit.

It is expressly agreed that this limited warranty shall be in lieu of all warranties of fitness and in lieu of the warranty of merchantability. Seller shall not be liable for any incidental or consequential damages that arise out of the installation, use or operation of Seller's product or out of the breach of any express or implied warranties."

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