INTENDED USE

The **Mouse anti- Ovomucoid (Trypsin Inhibitor) IgG** ELISA Kit is an immunoassay suitable for detecting and quantifying IgG antibody activity specific for Ovomucoid in serum or plasma. Other biological fluids, including tissue culture medium, may be validated for use. This kit is for research use only (RUO), not for diagnostic or therapeutic use.

GENERAL INFORMATION

Ovomucoid from chicken (also known as Gal d1) is a known allergen, along with 3 other egg white proteins: ovalbumin (Gal d 2), ovotransferrin (Gal d 3), and lysozyme (Gal d 4). Allergy to these proteins is one of the more frequent causes of food hypersensitivity in infants and young children. Ovomucoid-specific IgG has been shown in some cases to be more specific than egg-specific IgG for predicting food egg intolerance.

Ovomucoid is a trypsin inhibitor; the precursor is 210-aa secreted protein consisting of three tandem inhibitory domains (Kazal) homologous to pancreatic secretory trypsin inhibitor with reactive sites for serine proteinases.

PRINCIPLE OF THE TEST

The **Mouse Anti-Ovomucoid IgG** ELISA kit is based on the binding of anti-Ovomucoid IgG in samples to Ovomucoid antigen immobilized on the microwells; anti-Ovomucoid IgG antibody is detected by anti-IgG-specific antibody conjugated to HRP (horseradish peroxidase) enzyme. After a washing step, chromogenic substrate (TMB) is added and color is developed by the enzymatic reaction of HRP on the substrate which is directly proportional to the amount of anti- Ovomucoid IgG present in the sample. Stop Solution is added to terminate the reaction and A450nm is then measured using an ELISA reader. The presence of mouse IgG antibody in samples is determined relative to anti-Ovomucoid Calibrators.

PRODUCT SPECIFICATIONS

Specificity

Purified ovomucoid/trypsin inhibitor type III-O (ovoinhibitor free) from chicken egg white is used to coat the microwells; thus, no other antibody specificity is detectable in the assay. The Anti-mouse IgG HRP conjugate reacts specifically with mouse IgG class antibodies; IgA, IgE, and IgM antibody would not be measured above background signals.

Assay Sensitivity

The Ovomucoid antigen coating level, sample diluent, and HRP conjugate concentration are optimized to differentiate anti-Ovomucoid IgG from background (non-antibody) signal with mouse serum samples diluted 1:50 or lower.

KIT CONTENTS

The microtiter well plate and all other reagents, if unopened, are stable at 2-8ºC until the expiration date printed on the box label. Stabilities of the working solutions are indicated under Reagent Preparation.

To Be Reconstituted: Store as indicated.

Component	Preparation Instructions
Wash Solution Concentrate (100x) Cat. No. WB-100, 10ml	Dilute the entire volume 10ml + 990ml with distilled or deionized water into a clean stock bottle. Label as **Working Wash Solution** and store at 4ºC for long term and ambient temp. for short term.
Sample Diluent Concentrate (20x) Cat. No. SD-20T, 10ml	Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as **Working Sample Diluent** and store at 2-8ºC until the kit lot expires or is used up.
Anti-Mouse IgG-HRP Conjugate Concentrate (100x) Part: H-MsG.2a11, 0.15ml	Peroxidase conjugated anti-mouse IgG in buffer with detergents and antimicrobial as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of **Working Sample Diluent** is sufficient for 1 8-well strip. Use within the working day and discard. Return 100X to 2-8º C storage.

Ready For Use: Store as indicated on labels.

Component	Part	Amt	Contents
Ovomucoid Antigen Coated Strip Plate	610-221	8-well strips (12)	Coated with recombinant Ovomucoid protein, and post-coated with stabilizers.
Anti-Ovomucoid Calibrators			
1 U/ml 2.5 U/ml 5 U/ml 10 U/ml	610222B 610222C 610222D 610222E	0.65 ml 0.65 ml 0.65 ml 0.65 ml	Four (4) vials, each containing anti-Ovomucoid; in buffer with antimicrobial as stabilizers.
Anti-Ovomucoid Positive Control	610-222-PC	0.65 ml	Serum with anti-ovomucoid reactivity [Value range on vial label]
TMB Substrate	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
Stop Solution	80101	12 ml	Dilute sulfuric acid.

Materials Required But Not Provided:

- Pipettors and pipettes that deliver 100ul and 1-10ml.
- Disposable glass or plastic 5-15ml tubes for diluting samples and Anti-Mouse IgG HRP Concentrate.
- Stock bottle to store diluted Wash Solution; 0.2 to 1L.
- Distilled or deionized water to dilute reagent concentrates.
- Microwell plate reader at 450 nm wavelength and ELISA plate washer

ASSAY DESIGN AND SET-UP

Sample Collection and Handling

Serum and other biological fluids may be used as samples with proper dilution to avoid solution matrix interference. For **serum**, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature. For other samples, clarify the sample by centrifugation and/or filtration prior to dilution in Sample Diluent. If samples will not be assayed immediately, store refrigerated for up to a few weeks or frozen for long-term storage.

Antibody Stability

Dilution of serum into **Working Sample Diluent** (WSD) is recommended to stabilize antibody activity. This enhances reproducible sampling and stabilizes the antibody activity for years, stored refrigerated or frozen.

Assay Design

Review Interpretation of Results and Limits of the Assay (p. 5-7) before proceeding:

- Select the proper sample dilutions accounting for expected potency of positives and minimizing non-specific binding (NSB) and other matrix effects; for example, net signal for non-immune samples should be lower than the **1 U/ml Calibrator**. This is usually 1:25 or greater dilution for mouse serum/plasma with normal levels of IgG and IgM.
- Run a Sample Diluent **Blank**. This signal is an indicator of proper assay performance, especially of washing efficacy, and is used for net OD calculations, if required. Blank OD should be <0.3.
- Run the **Anti-Ovomucoid Positive Control**; value range is on the vial label.
- Run a set of **Calibrators**, which validate that the assay was performed to specifications: **10 U/ml** should give a high signal (>1.5 OD); **1 U/ml** should give a low signal which can be used to discriminate at the Positive/Negative threshold (see Interpretation of Results, p. 5).

Plate Set-up

Bring all reagents to room temperature (18-30º C) equilibration (at least 30 minutes).

- Determine the number of wells for the assay run. Duplicates are recommended, including 8 Calibrator wells and 2 wells for each sample control to be assayed.
- Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.
- Add 200-300ul Working Wash Solution to each well and let stand for about 1-5 minutes. Aspirate or dump the liquid and pat dry on a paper towel before sample addition.

Assay Procedure

ALL STEPS ARE PERFORMED AT ROOM TEMPERATURE. After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation.

1. 1st Incubation [100ul – 60 min; 4 washes]

- Add 100ul of calibrators, samples, and controls each to pre-determined wells.
- Tap the plate gently to mix reagents and incubate for 60 minutes.
- Wash wells 4 times and pat dry on fresh paper towels. As an alternative, an automatic plate washer may be used. Improper washes may lead to falsely elevated signals and poor reproducibility.

2. 2nd Incubation [100ul – 30 min; 5 washes]

- Add 100ul of diluted Anti-Mouse IgG HRP to each well.
- Incubate for 30 minutes.
- Wash wells 5 times as in step 1.

3. Substrate Incubation [100ul – 15 min]

- Add 100ul TMB Substrate to each well. The liquid in the wells will begin to turn blue.
- Incubate for 15 minutes in the dark, e.g., place in a drawer or closet.

Note: If your microplate reader does not register optical density (OD) above 2.0, incubate for less time, or read OD at 405-410 nm (results are valid).

4. Stop Step [Stop: 100ul]

- Add 100ul of Stop Solution to each well.
- Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.

5. Absorbance Reading

- Use any commercially available microplate reader capable of reading at 450nm wavelength. Use a program suitable for obtaining OD readings and data calculations if available.
- Read absorbance of the entire plate at 450nm using a single wavelength within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.

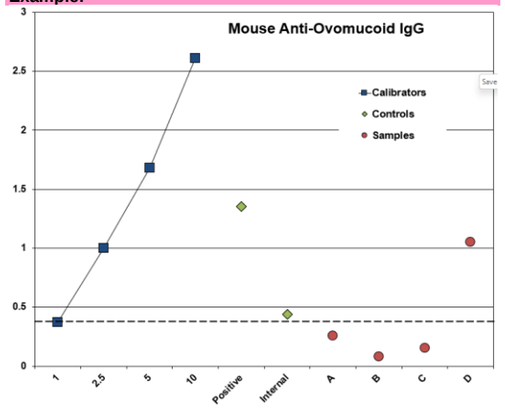
INTERPRETATION OF RESULTS

A. Antibody Activity Threshold Index

Compare Samples to **1 U/ml Calibrator** or **Internal Control**

= Positive/Negative Cut-off.

Example:



Results

The **sensitivity** of the assay to detect anti-Ovomucoid IgG, from either natural exposure or vaccination, is controlled so that the **1 U/ml Calibrator** represents a threshold OD for most true positives in mouse serum diluted to 1:25 or greater. Visual inspection of the data in the above graph shows the following:

Calibrators – dilution curve of an anti-Ovomucoid antibody, derived from Ovomucoid vaccination, shows the OD range of the assay; high value indicates optimal sensitivity of the assay.
1 U/ml: a 'Cut-off' line has been drawn to indicate a threshold distinguishing between **Positive/Negative**. The is not a clear-cut threshold, rather a low OD area that could represent either low positives or high background negatives.

Positive Control – serum showing reactivity to Ovomucoid; the value range is on the label. This Control may be used to gauge precision and to normalize between-assay variation.

Internal Control – a true positive from a mouse that represents the investigator's experience in distinguishing low positive from negative samples (not in kit). This should be run in each assay to supplement the 1 U/ml Calibrator for Positive/Negative discrimination purposes.

Samples A,B,C,D – 3 samples (B, C, D) are negative: below the threshold; 1 sample (A) is positive: clearly above the threshold.

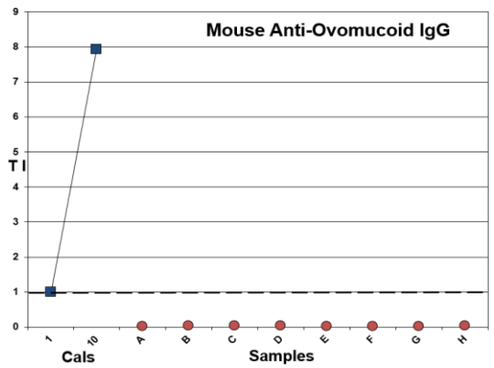
The 1 U/ml Calibrator can be used to calculate a **Threshold Index** that numerically discriminates Positive/Negative:

- Divide each Sample net OD by the 1 U/ml Calibrator net OD. Values above 1.0 are a measure of **Positive** Antibody Activity; below 1.0 are **Negative** for antibody.

INTERPRETATION OF RESULTS (cont)

Example:

Mouse Serum IgG
A panel of sera from laboratory mice was tested for anti-Ovomucoid IgG (1:50 dilution in Sample Diluent). **Threshold Index** was calculated using the **1** U/ml **Calibrator**.



Results

Anti-Ovomucoid IgG:
All of the mouse samples were negative (clearly below 1.0 TI).

Notes:

1. **Positives** may be due to prior exposure to ovomucoid, or an antigen with common epitopes, or from immunization.
2. When the **Positive Index** is **above 5.0,** using a dilution curve to calculate titer is a more accurate quantitation method (see Method C).
3. The **sensitivity** of the assay may be adjusted by changing the sample dilutions: a) increase dilution (e.g., 1:100) to lower the signals of borderline positives to negative; b) decrease dilution (e.g., 1:25) to convert borderline samples to positive. With the latter, the values of negatives may increase, so an alternative threshold should be considered using known negatives to develop a **Positive Index (**see below) or use an **Internal Control** (Page 5).

B. Positive Index
Experimental sample values may be expressed relative to the values of Control or Non-immune samples, by calculation of a **Positive Index**. One typical method is as follows:

1. Calculate the net OD mean + 2 SD of the Control/Non-immune samples = **Positive Index**.
2. Divide each sample net OD by the Positive Index. Values above 1.0 are a measure of **Positive** Antibody Activity; below 1.0 are **Negative** for antibody.

ASSAY PERFORMANCE

Method C. Titers from Sample Dilution Curves
The titer of antibody activity calculated from a dilution curve of each sample is recommended as the most accurate quantitative method. Best precision can be obtained using the following guidelines:

1. Use an OD value Index in the mid-range of the assay (2.0 – 0.5 OD); this provides the best sensitivity and reproducibility for comparing experimental groups and replicates. An arbitrary 1.0 OD is commonly used.
2. Prepare serial dilutions of each sample to provide a series that will produce signals higher and lower than the selected index. With accurate diluting, duplicates may not be required if at least 4 dilutions are run per sample.
3. A 5-fold dilution scheme is useful to efficiently cover a wide range which produces ODs both above and below 1.0 OD. The dilution scheme can be tightened to 3-fold or 2-fold for more precise comparative data.
4. The Calibrator values can be used to normalize inter-assay values.

Calculations

1. On a log scale of inverse of Sample Dilution as the x-axis, plot the OD values of the two dilutions of each positive sample having ODs above and below the OD value of the Index (arbitrary or selected Calibrator).
2. From a point-to-point line drawn between the two sample ODs, read the dilution value (x-axis) corresponding to the OD of the selected Index
   = **IgG Antibody Activity Units**

Limits of the Assay

- The assay detects and quantifies IgG antibodies directed to the ovomucoid protein. It may be possible for an allergen to chicken egg proteins to exist without detectable antibodies specific to the protein.
- Anti-COVM antibody levels may be below detection threshold related to the time course of the infection, e.g., too early for positive titer development.

PRECAUTIONS AND SAFETY INSTRUCTIONS

Calibrators, Sample Diluent, and Antibody HRP contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains dilute sulfuric acid. Follow good laboratory practices and avoid ingestion or contact of any reagent with skin, eyes, or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water. MSDS for TMB, sulfuric acid, and BND can be requested or obtained from the ADI website.

Instruction Manual No. 610-220-OMG

Mouse Anti-Ovomucoid IgG ELISA kit

Cat. #610-220-OMG

For the Detection and Quantitation of Anti-Ovomucoid (Trypsin Inhibitor) IgG in Mouse Serum/Plasma/Biological Samples

This kit is for research use only (RUO), not for therapeutic use.

ALPHA DIAGNOSTIC INTERNATIONAL

4638 N Loop 1604 W • San Antonio • Texas 78249 • USA

Phone (210) 561-9515 • Fax (210) 561-9544

Toll Free (800) 786-5777 Email: service@4adi.com

ELISA Kit Components	Amount	Part
Ovomucoid Antigen Coated Strip Plate	8-well strips (12)	610-221
Anti-Ovomucoid Positive Control	0.65 ml	610-222PC
Anti-Ovomucoid Calibrator 1 U/ml	0.65 ml	610-222B
Anti-Ovomucoid Calibrator 2.5 U/ml	0.65 ml	610-222C
Anti-Ovomucoid Calibrator 5 U/ml	0.65 ml	610-222D
Anti-Ovomucoid Calibrator 10 U/ml	0.65 ml	610-222E
Anti-Mouse IgG HRP Conjugate (100X)	0.15 ml	H-MsG.2a11
Sample Diluent (20x)	10 ml	SD20T
Wash Solution Concentrate (100X)	10 ml	WB-100
TMB Substrate	12 ml	80091
Stop Solution	12 ml	80101
Product Manual	1 ea	M-610-220-OMG