ELISA kits available from ADI (see details at the web site)

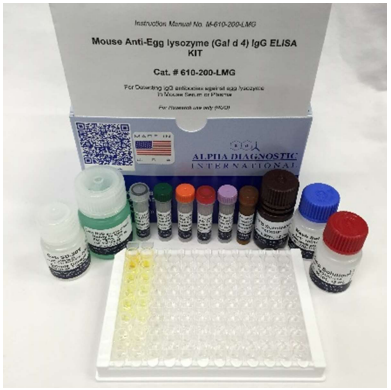
Catalog#	ProdDescription
610-200-LMG	Mouse Anti-Hen egg lysozyme (Gal d 4) IgG ELISA Kit, 96 tests, Quantitative
610-210-LMG	Human Anti-Hen egg lysozyme (Gal d 4) IgG ELISA Kit, 96 tests, Quantitative
610-220-OMG	Mouse Anti-chicken egg ovomucoid (Gal d 1, trypsin inhibitor) IgG ELISA Kit, 96
610-230-OMG	Human Anti-chicken egg ovomucoid (Gal d 1, trypsin inhibitor) IgG ELISA Kit, 96
610-240-TMG	Mouse Anti-chicken egg ovotransferrin (Gal d 3, IgG ELISA Kit, 96 tests,
610-250-TMG	Human Anti-chicken egg ovotransferrin (Gal d 3, IgG ELISA Kit, 96 tests,
600-105-OGG	Mouse Anti-Ovalbumin IgG (IgG specific) ELISA Kit, 96 tests, Quantitative
600-110-OG1	Mouse Anti-Ovalbumin IgG1 ELISA Kit, 96 tests, Quantitative
600-120-O2A	Mouse Anti-Ovalbumin IgG2a ELISA Kit, 96 tests, Quantitative
600-130-O2B	Mouse Anti-Ovalbumin IgG2b ELISA Kit, 96 tests, Quantitative
600-140-OG3	Mouse Anti-Ovalbumin IgG3 ELISA Kit, 96 tests, Quantitative
600-150-OGA	Mouse Anti-Ovalbumin IgA ELISA Kit, 96 tests, Quantitative
600-165-OGE	Mouse Anti-Ovalbumin IgE ELISA Kit, 96 tests, Quantitative
600-170-OGM	Mouse Anti-Ovalbumin IgM ELISA Kit, 96 tests, Quantitative
6050	Chicken Egg Ovalbumin ELISA Kit, 96 tests, Quantitative
610-110-OGE	Rat Anti-Ovalbumin IgE ELISA Kit, Quantitative
610-120-OGM	Rat Anti-Ovalbumin IgM ELISA Kit, 96 tests, Quantitative
620-100-OGG	Rabbit Anti-Ovalbumin IgG (total Ig's A+G+M) ELISA Kit, 96 tests, Quantitative
670-130-OVM	Monkey Anti-Ovalbumin Ig's ELISA Kit, 96 tests, Quantitative
670-140-OVG	Human Anti-Ovalbumin IgG ELISA Kit, 96 tests, Quantitative
670-145-OVM	Human Anti-Ovalbumin IgM ELISA Kit, 96 tests, Quantitative
670-150-OVE	Human Anti-Ovalbumin IgE ELISA Kit, 96 tests, Quantitative
AV-9315-1	Ovalbumin peptide OVA (257-264) class I MHC molecule
AV-9320-1	Ovalbumin peptide OVA (323-339) MHC class II peptide
AV-9335-10	Dinitrophenyl (DNP)-Ovalbumin (OVA) protein Conjugate
DNP55-N-10	Dinitrophenyl (DNP)-Ovalbumin (OVA) protein Conjugate
GSH15-N-100	Glutathione-Ovalbumin conjugate for ELISA
MA-20095	Mouse Monoclonal Anti-Ovalbumin (OVA) IgG, aff pure
NITT16-N	Nitrated egg ovalbumin protein for ELISA or controls (in PBS)
OVA11-A	Anti-chicken Egg Ovalbumin (OVA) IgG, aff pure
OVA11-AS	Anti-chicken egg ovalbumin IgG-agarose (aff matrix)
OVA11-S	Anti-chicken Egg Ovalbumin IgG
OVA12-R	Chicken Egg Ovalbumin purified protein
OVA13-M	Monoclonal Anti-chicken Egg Ovalbumin ascites (IgG1)
OVA14-S	Mouse polyclonal Anti-chicken Egg Ovalbumin Antiserum (IgA+G+M+E)
OVA15-N-1000	Chicken egg ovalbumin protein (ELISA, antigen, allergy grade)
OVA16-S	Rat polyclonal Anti-chicken Egg Ovalbumin serum (IgA+G+M+E)
RDT-8020-CO	TruStrip Rapid Ovalbumin Rapid Test cards, 25/pk
SP-53698-1	Ovalbumin (257-264) antigen peptide
610-200-LMG	Mouse Anti-Hen egg lysozyme IgG ELISA Kit, 96 tests, Quantitative
CELY14-S	Anti-Chicken Egg white lysozyme (muramidase) protein antiserum
CELY15-N	Chicken Egg white lysozyme (muramidase) protein
LYZM15-N-100	Lysozyme, Human Neutrophil
COVM13-S	Anti-Chicken Ovomucoid (trypsin inhibitor) protein antiserum
COVM15-N	Chicken Ovomucoid (trypsin inhibitor) protein
CECA15-S	Anti-Chicken Egg Conalbumin (ovotransferrin) protein
CECA16-N	Chicken Egg White Conalbumin (ovotransferrin) protein
CEGT11-S	Anti-Chicken Egg protein total (whole egg) antiserum
CEGT15-N	Chicken Egg proteins total (whole egg)
CEGW12-S	Anti-Chicken Egg white proteins total
CEGW15-N	Chicken Egg white proteins total

Instruction Manual No. M-610-200-LMG

Mouse Anti-Egg lysozyme (Gal d 4) IgG ELISA KIT

Cat. # 610-200-LMG, 96 Tests

For Detecting IgG antibodies against egg lysozyme
In Mouse Serum or Plasma



For Research use only (RUO)

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Toll Free (800) 786-5777

Email: service@4adi.com
Web Site: www.4adi.com

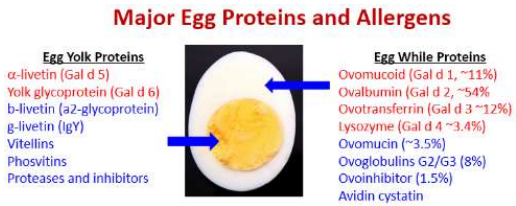
Mouse Anti-Egg Lysozyme IgG ELISA KIT Cat. # 610-200-LMG (96 tests)

Kit Components (96 tests)	qty
Purified Egg Lysozyme antigen coated strip plate, (8x12 strip or 96 wells) # 610201	1 plate
Anti-Lysozyme IgG **Std. A** (3 U/ml), 1 mL #610202A (clear cap)	1 vial
Anti- Lysozyme IgG **Std. B** (10 U/ml), 1 mL #610202B (green cap)	1 vial
Anti- Lysozyme IgG **Std. C** (30 U/ml), 1 mL #610202C (orange cap)	1 vial
Anti- Lysozyme IgG **Std. D** (90 U/ml), 1 mL #610202D (red cap)	1 vial
Anti- Lysozyme IgG Positive control, 1 mL #610203-PC (purple cap)	1 vial
All controls contain 0.02 % methylisothiazolone and 0.02 % bromonitrodioxane as preservative	
Anti-Mouse IgG-HRP Conjugate (100X), 0.15 ml, #H-MsG.211	1 vial
Sample/Conjugate Diluent (20X), 10ml, SD-20T	1 bottle
Low NSB sample diluent (TBTm) 30 ml (green Solution)	1 bottle
Wash buffer (100X) WB-100, 10 ml (blue cap)	1 bottle
TMB Substrate Solution, 80091, 12 ml (brown bottle)	1 bottle
Stop Solution, 80101, 12 ml # (red cap)	1 bottle
Complete Instruction Manual, M-610-200-LMG	1

Intended Use

Mouse anti-egg lysozyme IgG ELISA Kit is suitable for the detection and measurement of IgG antibody specific for lysozyme in mouse serum, plasma or other qualified biological samples from vaccinated, immunized and/or control animals. For research use only (RUO), not for diagnosis, cure or prevention of the disease.

General Information



Chicken egg is one of the most commonly available, economical foods. Eggs provide essential nutrients including high quality proteins, unsaturated fats, folate, and various vitamins. Eggs are 2nd most allergenic foods with approximately 2-3% of the population affected. Egg allergy is a type of food allergy. Both egg while and egg yolks have a number of allergens identified. Young children are particularly sensitive to food allergens. Egg allergy is the second most common food allergy in children (The most common is cows' milk allergy). The most severe food allergy reaction is called anaphylaxis and is an emergency requiring immediate attention and treatment with epinephrine. Most people who are allergic to hen's eggs have antibodies which react to one of four proteins in the egg white (ovomucoid, ovalbumin, ovotransferrin, and lysozyme). Ovomucoid, also called Gal d 1, is the most common target of immune system attack. The egg yolk contains several potential antigens: livetin, apovitillin, and phosvitin. Many common vaccines (Flu vaccine, yellow fever vaccine and MMR vaccine) are produced in eggs and may induce allergic reaction in sensitive individuals.

Lysozyme (LYS) of egg origin is being increasingly used as an antibacterial additive to prevent spoilage of cheese, wine or other foodstuff, as well as in medicinal products for respiratory tract infections and congestions, which do not usually declare its source, thus posing a risk for consumers allergic to hen egg. Hypersensitivity reactions to drugs containing

Interpretation of Results

There is no information available about the basal lysozyme antibody levels in mice. We recommend that the researchers establish basal levels for the control and vaccinated animals or establish their own know negative and positive controls.

Mouse Sample Testing

A random population of non-vaccinated, adult mice (Balb/c, mixed sex) were tested in the assay at 1:100 dilution.

Mouse samples	Mc136	Mc138	MC139	Mc140	Mc179	Mc180
OD @ 1:100	0.225	0.89	0.41	0.35	0.78	0.25

We recommend that users establish sample dilution that will give acceptable basal values for their animals. The actual test dilution can be varied from 1:100-1:500. Mice with no exposure to lysozyme should produce A450 values of <0.500 at a given dilution.

Quality Control

Blank values must be <0.500. Higher blank values are usually from inefficient washing. In case of high blank, re-run the standards and blanks until satisfactory values are established.

Highest standard (D) values must be >1.00
Anti-mouse lysozyme IgG Positive control values should be >0.6 (0.800-1.4 range)

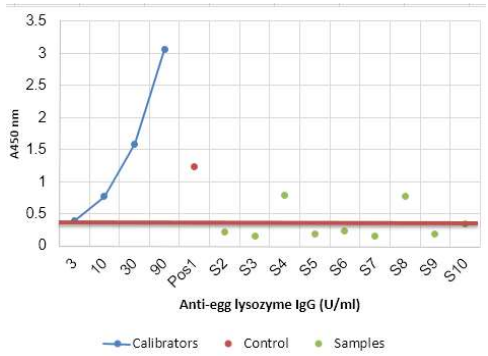
If criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls. In case of any deviation the following technical issues should be proven (reagents, protocol, equipments, etc).

References: Hviid A (2008) Lancet 371, 932; Bedford H (2005) Nurs. Times 101, 3; 39; Peltola H (2007) Clin. Infec. Dis. 45, 459; Tardieu M (1982) Scinece 215, 419-421; Xu P (2013) J. Virol. 87, 8155-8168; Cusi MG (2001) Arch. Virol. 146:1241–1248; Tsurudome M (1987) Arch. Virol. 97:167–179; Vandermeulen C, (2010) Immunology 131:33–39;

WORKSHEET OF A TYPICAL ASSAY

Wells	Stds/samples	U/mL	Mean A450	Net Values (minus blank)
A1, A2	**Blanks (**	0	0.100	0
B1, B2	Standard A	3	0.490	0.39
C1, C2	Standard B	10	0.869	0.769
D1, D2	Standard C	30	1.69	1.59
E1, E2	Standard D	90	3.106	3.06
F1, F2	**Positive control**		1.3	0.39
G1, G2	Sample 1			
H1, H2	Sample 2			

NOTE: These data are for demonstration purpose only. It must not be used to determine the sample results. Actual values may differ from the above and may be lot specific. Lot specific and the values obtained for a given run should be used for calculation of sample values.



610-200-LMG-ELISA-Graph/ /nase1

CALCULATION OF RESULTS

The mean values for the measured absorptions are calculated after subtraction of the blank values from the controls and standards.

The obtained OD of the standards (y-axis, linear) are plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic graph paper or using an automated method. A good fit is provided with cubic spline, 4 parameter logistics or Logit-Log. For the calculation of the standard curve apply each signal of the standards (one obvious outlier of duplicates might be omitted and the more plausible single value might be used). The concentration of the samples can be read from the standards curve. The initial dilution has been taken into consideration when reading the results from the graph. Results of samples of higher predilution have to be multiplied with the dilution factor. Samples showing concentrations above the highest standard have to be diluted as described in “Assay Procedure” and reassayed.

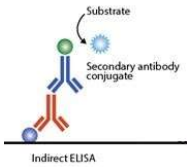
LYS have been described that, in some cases, happen to occur in children that had never intentionally eaten egg and either ignore they are sensitized to egg proteins or react at the first ingestion.

Lysozymes (185-aa, ~15 kda), also known as muramidase or N-acetylmuramide glycanhydrolase, are glycoside hydrolases. These are enzymes (EC 3.2.1.17) that damage bacterial cell walls by catalyzing hydrolysis of 1,4-beta-linkages between N-acetylmuramic acid and N-acetyl-D-glucosamine residues in a peptidoglycan and between N-acetyl-D-glucosamine residues in chitodextrins. Large amounts of lysozyme can be found in egg white. Different lysozymes are found in many organisms, from bacteriophages to mammals, and in general, they show little sequence similarity to each other. Lysozyme from some common species have the following protein conservations: Goose (185-aa), chicken (80%), human (44%), and mouse (43%).

Egg allergies are currently diagnosed based on a patient’s clinical history, a physical examination, a skin prick test, and the presence of immunoglobulin (Ig) E antibodies specific for egg white.

Lysozyme has been shown to possess certain dominant T-cell epitope that may enhance antibodies or autoantibody production (47-61, 74-88, 107-116).

PRINCIPLE OF THE TEST



Alpha Diagnostic’s anti-lyozyme IgG antibody test kit is based on the principle of the indirect ELISA. Lysozyme antigen is bound on the surface of the microtiter strips. Diluted samples or ready-to-use standards are pipetted into the wells of the microtiter plate. A binding between the IgG antibodies of the serum and the immobilized lysozyme antigen takes place. After a one hour incubation at room temperature, the plate is rinsed with diluted wash solution, in order to remove unbound material. Anti-IgG peroxidase conjugate is added and incubated. After a further washing step, the substrate (TMB) solution is pipetted and incubated, inducing the development of a blue dye in the wells. The color development is terminated by the addition of a stop solution, which changes the color from blue to yellow. The resulting dye is measured at 450 nm in an ELISA reader. The concentration of the IgG antibodies is directly proportional to the intensity of the color.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (5µl, 100µl, 500µl) and multichannel pipet with disposable plastic tips. Bidistilled water, reagent troughs, Orbital shaker, plate washer (recommended) and ELISA plate Reader (450nm).
Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site.
TMB (substrate), H2SO4 (stop solution), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).

http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10

SPECIMEN COLLECTION AND HANDLING

Principally serum or plasma (EDTA, heparin) can be used for the determination. Serum is separated from the blood, which is aseptically drawn by venipuncture, after clotting and centrifugation. The serum or plasma samples can be stored refrigerated (2-8°C) for up to 48 hours, for a longer storage they should be kept at -20 °C. The samples should not be frozen and thawed repeatedly. Lipemic, hemolytic or bacterially contaminated samples can cause false positive or false negative results. For the performance of the test the samples (not the standards) have to be diluted 1:500 (or more, see below) with 1X sample diluent.

Sample Dilutions

An initial test dilution of **1:100** is recommended for most mice. Basal lysozyme antibody values may differ due to strains, age, sex, and potential or intentional exposure to lysozyme antigen. Therefore, final test dilution can be varied from 1:100-1:500. Vaccinated or immunized samples should be tested at higher dilutions or until sample values are within the range of the standards.

Sample Stability

Initial dilution of serum (1:10; or 5 ul serum into 45 ul diluent) into **1x Sample Diluent** is recommended to stabilize antibody activity. This enhances reproducible sampling, and stabilizes the antibody activity for years, stored refrigerated or frozen. Further test dilution (1:500 or more) should be performed the same day as the assay. Final test dilutions should be prepared in LowNSB diluent (green diluent). Do not store test sample dilutions beyond the assay date.

Example:

Initial (1/10): **5**ul serum + **45**ul 1X sample dilution (store at 4oC)
Further (1/100): **25** ul initial (1/10) + **225** ul **Low NSB diluent** (green diluent) (use 100 ul x 2 for testing)

REAGENTS PREPARATION

1. **Dilute Wash buffer** 1:100 with distilled water (10 ml stock in 990 ml water). Store diluted buffer at 4oC for 1 month. (If during the cold storage crystals precipitate, the concentrate should be warmed up at 37 degrees C for 15 minutes.
2. **Dilute 100x HRP Conjugate**- 100x conjugate in 1X sample diluent (10 ul stock conjugate in 990 ul diluent). Do not use any other diluent to dilute the enzyme conjugate. Make 1 ml per 8-well strip or 10 ml for the entire plate. Do not store unused 1X conjugate and prepare only in require amounts.

All reagents must be at room temperature prior to their use.

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8^{o}C until the expiration date printed on the label. The whole kit stability is usually 6 months from the date of shipping under appropriate storage conditions. The unused portions of the standards should be stored at 2-8^{o}C or stored frozen in small aliquots and should be stable for 3 months.

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).* **Prepare 1X Sample diluent, Wash buffer, and antibody conjugate**.

Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag. **All samples should be diluted 1:100 (see page 3)**. It is recommended to prepare a parallel replica plates containing all sample for quick transfer to the coated plate. **Standard and control are provided ready to use.**

1. Label or mark the microtiter well strips to be used on the plate.

2. Pipet **100 ul of sample diluent (buffer blank),** Standards, positive controls, and samples (diluted 1:100) into appropriate wells in *duplicate*. See worksheet of a typical set-up on page 5. Cover the plate, mix gently for 5-seconds and incubate at room temp for 60 min.

3. Aspirate the well contents and blot the plate on absorbent paper. Immediately, **wash the wells 3 times** with 250-300 ul of 1X wash buffer. We recommend using an automated ELISA plate Washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.

4. Add **100 ul anti-IgG-HRP conjugate** to all wells leaving one empty for the substrate blank. Mix gently for 5-10 seconds. Cover the plate and incubate for **30 minutes** at room temp (25-28oC).

5. Wash the wells 3 times as in step 3.

6. Add **100 ul TMB substrate solution**. Mix gently for 5-10 seconds. Cover the plate and incubate for **20 minutes** at room temp. Blue color develops in positive controls and samples.

7. Stop the reaction by adding **100 ul of stop solution** to all wells. Mix gently for 5-10 seconds to have uniform color distribution (blue color turns yellow).

8. Measure the **absorbance at 450 nm** using an ELISA reader within 15 min.

NOTES

Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 4^{o}C. Do not touch the bottom of the wells.