Chicken IgM ELISA Kit

Cat. No. E33-102
Lot No. 260622

Components Supplied

- **Chicken IgM Pre-Coated 96-well Strip Plate,** 1 each
- **Chicken IgM Standard**, 500 ng/vial, 2 each
- **Chicken IgM Detection Antibody,** 12 ml
- **10X Dilution Buffer B**, 25 ml
- **HRP Solution A,** 12 ml
- **TMB Substrate,** 12 ml
- **Stop Solution,** 12 ml
- **20X Wash Buffer,** 50 ml
- **Sealing Tape,** 6 sheets

Introduction

Enzyme linked immunosorbent assay (ELISA) for the detection of Chicken IgM in serum, plasma, or other biological fluids. Kit contains sufficient components to quantitate Chicken IgM protein concentration in up to 40 samples, tested in duplicate.

Background

Avian antibodies have been recognized for decades and offer many advantages to mammalian antibodies in research and diagnostics. The only avian species in which antibodies are highly defined and easily accessible are the chicken. In mammals, immunoglobulins are classified into five groups: IgA, IgD, IgE, IgG and IgM. Chickens possess three principle classes of immunoglobulins: IgA, IgY (IgG) and IgM (Lebacq-Verheyden, 1974). Moreover, the structures of the corresponding immunoglobulins are significantly different, namely in the number of constant domains within the heavy chains. While avian IgY (IgG) is comprised of four constant domains, mammalian IgG contains only three (Hodek, 2003).

Chicken IgM is the first antibody observed after primary immunization and IgM is expressed on the surface of most chicken B cells. During the course of an immune response, IgM is produced during the first week post-infection (Rathnapraba, 2007). The average concentration of IgM in chicken serum is about 2.55 mg/ml. IgM is also known to be transferred with other proteins in the oviduct into the egg white but in very low amounts.

Principle of the Assay

This kit is based on a sandwich ELISA. Chicken IgM present in the test sample is captured by anti-chicken IgM antibody that has been pre-adsorbed on the surface of microtiter wells. After sample binding, unbound proteins and molecules are washed off, and a biotinylated detection antibody is added to the wells to bind to the captured IgM. A strepavidin-conjugated horseradish peroxidase (SA-HRP) is then added to catalyze a colorimetric reaction with the chromogenic substrate TMB (3,3',5,5'-tetramethylbenzidine). The colorimetric reaction produces a blue product, which turns yellow when the reaction is terminated by addition of dilute sulfuric acid. The absorbance of the yellow product at 450 nm is proportional to the amount of IgM analyte present in the sample and a four-parameter standard curve can be generated. The IgM concentrations in the test samples can then be quantified by interpolating their absorbance from the standard curve generated in parallel with the samples. After factoring sample dilutions, the IgM concentrations in the original sample can finally be calculated.

Procedure Overview

1. Add 100 µl of standard or sample to well.
   Note: Run each standard or sample in duplicate.



2. Cover plate and incubate at room temperature (20-25°C) for 1 hour.



3. Wash plate FOUR times.



4. Add 100 µl of Chicken IgM Detection Antibody to each well.



5. Cover plate and incubate at room temperature for 1 hour.



6. Wash plate FOUR times.



7. Add 100 µl of HRP Solution A to each well.



8. Cover plate and incubate at room temperature for 30 minutes.



9. Wash plate FOUR times.



10. Add 100 µl of TMB Substrate Solution to each well.



11. Develop the plate in the dark at room temperature for 30 minutes.



12. Stop reaction by adding 100 µl of Stop Solution to each well.



13. Measure absorbance on a plate reader at 450 nm.

Additional Materials Required

- Ultrapure water
- Precision pipettors, with disposable plastic tips
- Polypropylene or polyethylene tubes to prepare standard and samples − do not use polystyrene, polycarbonate or glass tubes
- A container to prepare 1X Dilution Buffer B
- A container to prepare 1X Wash Buffer
- A wash bottle or an automated 96-well plate washer
- Disposable reagent reservoirs
- A standard microtiter plate reader for measuring absorbance at 450 nm

Precautions

- Store all reagents at 2-8ºC. Do not freeze reagents.
- All reagents must be at room temperature (20-25°C) before use.
- Vigorous plate washing is essential.
- Use new disposable pipette tips for each transfer to avoid cross-contamination.
- Use a new adhesive plate cover for each incubation step.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Avoid microbial contamination of reagents and equipment. Automated plate washers can easily become contaminated thereby causing assay variability.
- Take care not to contaminate the TMB Solution. Do not expose TMB Substrate solution to glass, foil, or metal. If the solution is blue before use, DO NOT USE IT.
- Individual components may contain preservatives. Wear gloves while performing the assay. Please follow proper disposal procedures.

Standard and Sample Handling and Preparation

1X Dilution Buffer B Preparation

- Prepare 1X Dilution Buffer B by diluting 10X Dilution Buffer B in ultra pure water. For example, if preparing 250 ml of 1X Dilution Buffer B, dilute 25 ml of 10X Dilution Buffer B into 225 ml of ultrapure water. Mix well. Store reconstituted 1X Dilution Buffer B at 2-8ºC for up to six (6) months. Do not use 1X Dilution Buffer B if it becomes visibly contaminated during storage.

Standard Preparation

1. Reconstitute the 500-ng Chicken IgM standard vial with 1.0 ml of 1X Dilution Buffer B to achieve a final concentration of 500 ng/ml. Mix well.
2. Label seven (7) tubes, one for each standard curve point: 167, 55.6, 18.5, 6.17, 2.06, 0.69, and 0 ng/ml. The original vial of re-suspended standard solution represents the top standard at 500 ng/ml.
3. Add 300 µl of 1X Dilution Buffer B into each of the seven tubes.
4. Serially dilute 1:3 by adding 150 µl of the 500 ng/ml standard into the first tube containing 300 µl of 1X Dilution Buffer B. Mix well. Continue the dilution by adding 150 µl of the previous standard into 300 µl of 1X Dilution Buffer B in the next tube until the sixth tube (0.69 ng/ml).
5. The seventh tube containing 300 µl of 1X Dilution Buffer B serves as the zero standard value or blank.

- Using this dilution scheme, only 350 µl of the re-suspended standard solution should be used. The remaining standard solution can be used for another assay within the same day. Otherwise, use the second vial of lyophilized standard.

Sample Handling

- This ELISA assay can be used for serum, plasma, and other biological fluids.
- All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions when handling and disposing of infectious agents.
- 100 µl of sample or standard is required per well.

- Samples must be assayed in duplicate each time the assay is performed.
- Store samples to be assayed within 24 hours at 2-8ºC. For long-term storage, aliquot and freeze samples at -70°C. Avoid repeated freeze-thaw cycles when storing samples.
- If particulate is present in samples, centrifuge prior to analysis.
- If the integrity of the sample is of concern, make a note on the Plate Template and interpret results with caution.

Sample Preparation

- The dilution schemes indicated below are only suggestions. Dilutions should be based on the expected concentration of the unknown sample such that the diluted sample falls within the dynamic range of the standard curve. Prepare one or more dilutions of the sample in 1X Dilution Buffer B using the same serial dilution technique described below until the desired concentration is obtained.
- Serum and plasma – Recommended starting dilution is 1:10,000. With this large dilution one must strive for accurate pipetting. A typical dilution scheme starts by adding 10 µl of plasma into 990 µl of 1X Dilution Buffer B to give a 1:100 dilution. This is repeated by adding 10 µl of the 1:100 diluted sample to 990 µl of 1X Dilution Buffer B to give 1:10,000 dilution.

1X Wash Buffer Preparation

- Prepare 1X Wash Buffer by diluting 20X Wash Buffer in ultra pure water. For example, if preparing 1 L of 1X Wash Buffer, dilute 50 ml of 20X Wash Buffer into 950 ml of ultrapure water. Mix well. Store reconstituted 1X Wash Buffer at 2-8ºC for up to six (6) months. Do not use 1X Wash Buffer if it becomes visibly contaminated during storage.

Assay Procedure

Sample Incubation

- Determine the number of strips required. Leave these strips in the plate frame. Place unused strips in the foil pouch with desiccant and seal tightly. Store unused strips at 2-8°C. After completing assay, keep the plate frame for additional assays.

- Use a Plate Template to record the locations of the standards and unknown samples within the wells.

1. Add 100 µl of appropriately diluted standards or samples to each well. Run each standard, sample, or blank in duplicate.
2. Carefully cover wells with a new adhesive plate cover. Incubate for one (1) hour at room temperature, 20-25°C.
3. Carefully remove adhesive plate cover, discard plate contents and wash FOUR times with 1X Wash Buffer as described in the Plate Washing section.

Plate Washing

1. Gently squeeze the long sides of plate frame before washing to ensure all strips remain securely in the frame.
2. Empty plate contents. Use a squirt wash bottle to vigorously fill each well completely with 1X Wash Buffer, then empty plate contents. Repeat procedure three additional times for a total of FOUR washes. Blot plate onto paper towels or other absorbent material.

   Note: For automated washing, aspirate plate contents from all wells and flood wells with 1X Wash Buffer. Repeat procedure three additional times for a total of FOUR washes. Additional washes may be necessary. Blot plate onto paper towels or other absorbent material.

   Take care to avoid microbial contamination of equipment. Automated plate washers can easily become contaminated thereby causing assay variability.

Detection Antibody Incubation

- Only remove the required amount of Detection Antibody reagent for the number of strips being used.

1. Add 100 µl of Detection Antibody to each well containing standard, sample or blank. Mix well by gently tapping the plate several times.
2. Carefully attach a new adhesive plate cover. Incubate plate for one (1) hour at room temperature, 20-25°C.
3. Carefully remove the adhesive plate cover, discard plate contents and wash FOUR times with 1X Wash Buffer as described in the Plate Washing section.

HRP Solution A Incubation

- Only remove the required amount of HRP Solution A for the number of strips being used.

1. Add 100 µl of HRP Solution A to each well containing sample or blank.
2. Carefully attach a new adhesive plate cover. Incubate plate for 30 minutes at room temperature, 20-25°C.
3. Carefully remove the adhesive plate cover, discard plate contents and wash FOUR times with 1X Wash Buffer as described in the Plate Washing section.

TMB Substrate Incubation and Reaction Stop

- Only remove the required amount of TMB Substrate Solution and Stop Solution for the number of strips being used.
- Do NOT use a glass pipette to measure the TMB Substrate Solution. Do NOT cover the plate with aluminum foil or metalized mylar. Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate Solution. If the solution is blue before use, DO NOT USE IT!

1. Add 100 µl of TMB Substrate Solution into each well.
2. Allow the enzymatic color reaction to develop at room temperature (20-25ºC) in the dark for 30 minutes. Do NOT cover plate with a plate sealer. The substrate reaction yields a blue solution.
3. After 30 minutes, stop the reaction by adding 100 µl of Stop Solution to each well. Tap plate gently to mix. The solution in the wells should change from blue to yellow.

Absorbance Measurement

Note: Evaluate the plate within 30 minutes of stopping the reaction.

1. Wipe underside of wells with a lint-free tissue.
2. Measure the absorbance on an ELISA plate reader set at 450 nm.

Calculation of Results

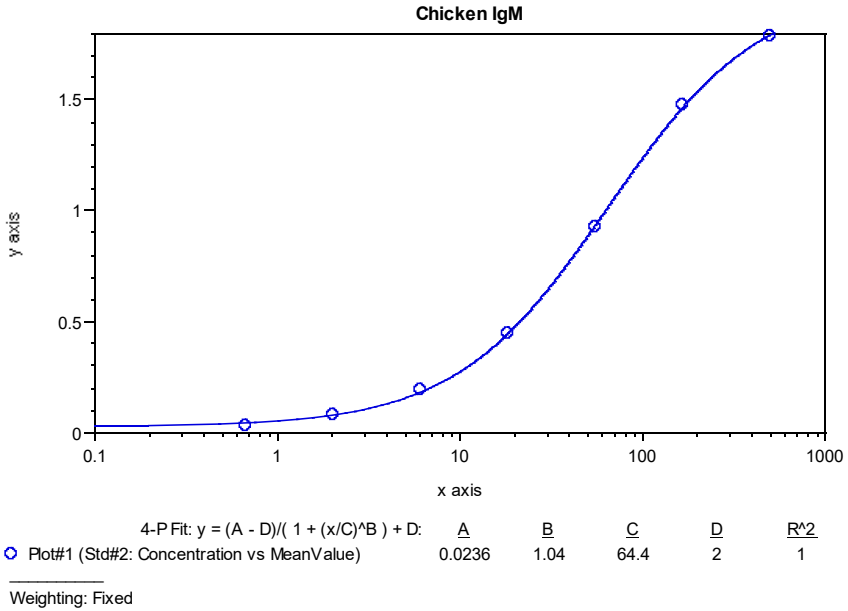
- Duplicate absorbance values should be within 10% of each other. Care should be taken when interpreting data with differences in absorbance values greater than 10%.

1. Prepare a standard curve to determine the amount of Chicken IgM in an unknown sample. Plot the average absorbance obtained for each standard concentration on the vertical (Y) axis versus the corresponding Chicken IgM concentration on the horizontal (X) axis using graph paper or curve-fitting software.
2. Calculate the Chicken IgM concentration in unknown samples using the prepared standard curve. Determine the amount of Chicken IgM in each unknown sample by noting the Chicken IgM concentration (X axis) that correlates with the absorbance value (Y axis) obtained for the unknown sample.
3. If the sample was diluted, multiply the Chicken IgM concentration obtained by the dilution factor to determine the amount of Chicken IgM in the undiluted sample.

Performance Characteristics

Typical Standard Curve

- This typical standard curve was generated using the Chicken IgM ELISA Kit Protocol. This standard curve is for demonstration only. A standard curve must be generated for each assay.



Assay Range: 0.69 – 500 ng/ml

- Suggested standard curve points are 500 ng/ml, 167 ng/ml, 55.6 ng/ml, 18.5 ng/ml, 6.17 ng/ml, 2.06 ng/ml, 0.69 ng/ml, and 0 ng/ml.

Warranty

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Background References

1. Lebacq-Verheyden, Vaerman, J.P., and J. F. Heremans (1974). Quantification and Distribution of Chicken Immunoglobulins IgA, IgM, and IgG in Serum and Secretions. *Immunology* 27: 683.
2. Hodek, Peter and Maria Stiborova (2003). Chicken Antibodies – Superior Alternative to Conventional Immunoglobulins. *Proc Indian Natn Sci Acad*. 4: 461.
3. Rathnapraba, S., Raj, G. Dhinakar, and V. Thiagarajan (2007). Development of Monoclonal Antibodies against Chicken IgM and its Application in Immunity Studies. *Indian Journal of Biotechnology*. 6: 187.

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Plate Templates

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
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	1	2	3	4	5	6	7	8	9	10	11	12
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