EAGLE BIOSCIENCES

Chicken IgY ELISA

Catalog Number:
IGG99-K01 (1 x 96 wells)
For Research Use Only. Not for use in diagnostic procedures.
v. 1.0

EAGLE BIOSCIENCES, INC.
20A Northwest Blvd., Suite 112, Nashua, NH 03063
Phone: 617-419-2019 Fax: 617-419-1110
WWW.EAGLEBIO.COM

Introduction

The Eagle Biosciences Chicken IgY ELISA Assay Kit is designed for the quantitative determination of chicken IgY in complex samples (serum or other biological samples). The Chicken IgY ELISA Assay Kit is for research use only and should not be used in diagnostic procedures.

Chicken IgG and chicken IgY are two different names for the same antibody. Immunoglobulin Y (abbreviated as IgY) is a type of immunoglobulin which is the major antibody in bird, reptile, and lungfish blood. It is also found in high concentrations in chicken egg yolk. As with the other immunoglobulins, IgY is a class of proteins which are formed by the immune system in reaction to certain foreign substances, and specifically recognize them. IgY is often mislabelled as Immunoglobulin G (IgG) in older literature, and sometimes even in commercial product catalogues due to its functional similarity to mammalian IgG and Immunoglobulin E (IgE).

Principle of the Assay

The determination of chicken IgY is carried out as direct sandwich ELISA. An antibody specific for chicken IgY has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IgY present is bound. After washing away any unbound substances, an enzyme-linked antibody is added. Following a wash, a substrate solution is added to the wells and color develops in proportion to the amount of antibody conjugate. The absorption at 450 nm is proportional to the IgY concentration.

Precautions

- Store the Chicken IgY ELISA Assay Kit at 2-8 °C.
- The Chicken IgY ELISA Assay Kit is for research use only. Not for use in diagnostic procedures.
- Do not use the reagents beyond the expiration date marked on box label.
- Please read the instructions carefully before using the Chicken IgY ELISA Assay Kit.
- Do not mix reagents from different lots.
- Some components of this Chicken IgY ELISA Assay Kit contain Thimerosal, a mercury containing compound. Follow routine precautions for handling hazardous chemicals. Avoid contact with skin and mucous membranes when handling reagents, which contain preservatives (see materials provided). Wash thoroughly with water in case of contact and possibly look up a doctor.
- The stop solution contains 0.5 M sulphuric acid. Wash thoroughly with water in case of contact with skin. In case of contact with eyes rinse with much water and look up a doctor.
- Do not allow the wells to become dry once the Chicken IgY ELISA Assay has begun.

Other supplies required

- Deionized or distilled water
- Graduated cylinder
- Micropipettes, multipipette
- Microplate reader

Preparation of reagents and samples

- Bring all reagents of the Chicken IgY ELISA Assay Kit to room temperature before use. If crystals have formed, mix gently until the crystals have completely dissolved.

- The microplate strips are ready to use. Remove excess strips (breakable) from the frame, reseal in the bag with the desiccant and store at 2-8 °C

- Dilute the wash buffer with deionized or distilled water **1:10** (e. g. 40 ml + 360 ml water). The diluted solution is stable for 30 days at 2-8 °C.

- Use the Standard concentrate to produce a 1:2-dilution series with diluent (e. g. 250 µl + 250 µl diluent):

Standard	Preparation	Conc. (ng/ml)
S 7	standard conc. Undiluted	200
S 6	S 7 1:2 diluted	100
S 5	S 6 1:2 diluted	50
S 4	S 5 1:2 diluted	25
S 3	S 4 1:2 diluted	12.5
S 2	S 3 1:2 diluted	6.25
S 1	S 2 1:2 diluted	3.125

- Dilute the samples with diluent. If samples generate values outside the standard curve, the dilution factor may be varied. To exclude matrix effects the dilution factor should be at least 1:25.

Assay procedure

It is recommended that all samples and standards be assayed in duplicate.

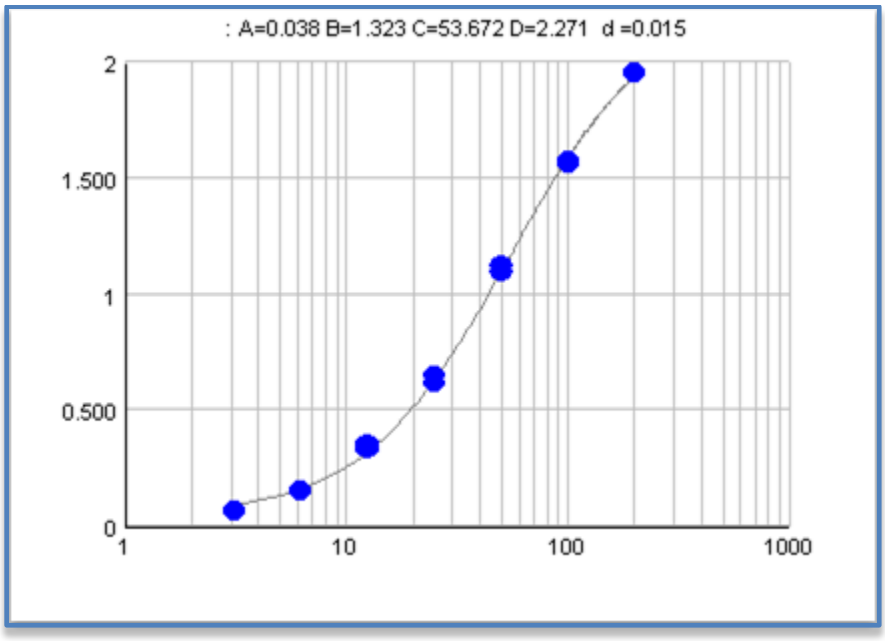
1. Prepare all reagents, standard curve and samples as directed in the previous section.
2. Pipette 100 µl of samples, standards, positive control or diluent (as negative control) into the wells.
3. Seal wells with adhesive strip and incubate for 1 hour at room temperature with shaking.
4. Aspirate fluid from wells and wash three times with 300 µl wash buffer. After the last wash, invert the plate and tap on a clean paper towel.
5. Add 100 µl of HRP conjugate to each well.
6. Seal wells with adhesive strip and incubate for 1 hour at room temperature with shaking.
7. Repeat the wash as in step 4.
8. Dispense 100 µl of TMB substrate solution into each well.
9. Incubate for 10 minutes at room temperature in the dark.
10. Add 100 µl of stop solution to each well.
11. Determine the absorbance within 30 minutes at 450 nm. A reference wavelength of 620 nm/690 nm is recommended.

Calculation of results

Create a standard curve for the Chicken IgY ELISA Assay Kit using computer software capable of generating a curve fit (e.g. x-axis: log, IgG concentration; y-axis: linear, absorbance; four parameter curve fit). As an alternative, draw a standard curve on semi-log paper (x-axis: log, IgG concentration; y-axis: linear, absorbance). The IgG concentrations can be calculated from the standard curve. The calculated concentrations must be multiplied by the sample dilution factor.

If the absorbance of some samples is outside the standard curve a subsequent determination with changed dilutions will provide a proper result.

Typical Standard Curve:



Performance Characteristics

- Standard curve:
  7 standards between 3.125 ng/ml and 200 ng/ml
- Sensitivity:
  80 ng/ml (sample dilution 1:25)
- Sample materials:
  Protein solutions, serum, biological fluids
- Intraassay precision (CV):
  (n=10)
  at 50.8 ng/ml: 3.0%
  at 122.6 ng/ml: 6.6%

Materials provided:

Number of determinations	1x96 determinations
Microplate strips, antibody coated	12 x 8
Wash buffer, 10fold conc. ✦	50 ml
Diluent, ready to use ✦	100 ml
Standard concentrate, 200 ng/ml ✦	2 ml
Positive control, ready to use ✦	1 ml
Anti-IgG(chicken)-Ab., HRP conjugate, ready to use ✦	12 ml
TMB substrate, ready to use	12 ml
Stop solution, ready to use (0.5 M sulphuric acid)	12 ml

✦: contains Thimerosal

Assay procedure summary:

A. Preparation
1. Bring all reagents to room temperature
2. Dilute wash buffer 1:10
3. Prepare the standard curve from a 1:2-dilution series of standard concentrate with diluent
4. Dilute samples with diluent

B. Performance
1. Pipette 100 µl of samples, standards, controls into the wells
2. Incubate for 1 hour at room temperature with shaking
3. Wash three times with 300 µl of wash buffer
4. Add 100 µl of HRP conjugate to each well
5. Incubate for 1 hour at room temperature with shaking
6. Wash three times with 300 µl of wash buffer
7. Dispense 100 µl of TMB substrate solution
8. Incubate for 10 minutes at room temperature in the dark
9. Add 100 µl of stop solution
10. Measure absorption at 450 nm

Warranty Information

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

Eagle Biosciences makes no warranties, either expressed or implied, except as provided herein, including without limitation thereof, warranties as to marketability, merchantability, fitness for a particular purpose or use, or non-infringement of any intellectual property rights. In no event shall the company be liable for any indirect, incidental, or consequential damages of any nature, or losses or expenses resulting from any defective product or the use of any product. Product(s) may not be resold, modified, or altered for resale without prior written approval from Eagle Biosciences, Inc.

For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.