Chicken IgA ELISA Assay Kit

Catalog Number:
CKA99-K01 (1 x 96 wells)
For Research Use Only. Not for use in diagnostic
v. 2 (25 SEP 23)

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INTENDED USE

The Chicken IgA ELISA Assay Kit is a highly sensitive two-site enzyme linked immunoassay (ELISA) for measuring IgA in biological samples of Chickens.

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

PRINCIPLE OF THE ASSAY

The principle of the double antibody sandwich ELISA is represented in Figure 1. In this assay the IgA present in samples reacts with the anti-IgA antibodies which have been adsorbed to the surface of polystyrene microtitre wells. After the removal of unbound proteins by washing, anti-IgA antibodies conjugated with horseradish peroxidase (HRP), are added. These enzyme-labeled antibodies form complexes with the previously bound IgA. Following another washing step, the enzyme bound to the immunosorbent is assayed by the addition of a chromogenic substrate, 3,3',5,5'-tetramethylbenzidine (TMB). The quantity of bound enzyme varies directly with the concentration of IgA in the sample tested; thus, the absorbance, at 450 nm, is a measure of the concentration of IgA in the test sample. The quantity of IgA in the test sample can be interpolated from the standard curve constructed from the standards, and corrected for sample dilution.

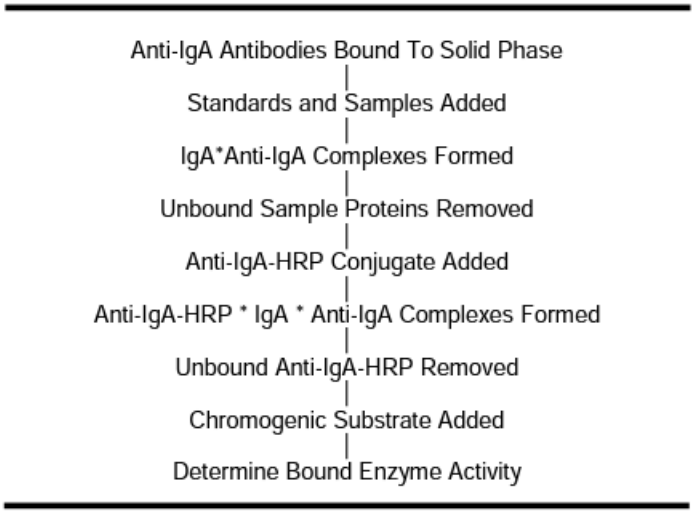

Figure 1.

MATERIALS PROVIDED

1. **Diluent Concentrate**

   (Running Buffer) One bottle containing 50 ml of a 5X concentrated diluent running buffer.

2. **Wash Solution Concentrate**

   One bottle containing 50 ml of a 20X concentrated wash solution.

3. **Enzyme-Antibody Concentrate**

   100X One vial containing 150 µL of affinity purified antiChicken IgA antibody conjugated with horseradish peroxidase in a stabilizing buffer.

4. **Chromogen-Substrate Solution**

   One vial containing 12 mL of 3,3',5,5'tetramethybenzidine (TMB) and hydrogen peroxide in citric acid buffer at pH 3.3.

5. **Stop Solution**

   One vial containing 12 ml 0.3 M sulfuric acid.
   WARNING: Avoid contact with skin.

6. **Anti-Chicken lgA ELISA Microplate**

   Twelve removable eight (8) well micro well strips in well holder frame. Each well is coated with affinity purified anti-Chicken IgA.

7. **Chicken lgA Calibrator**

   One vial containing Chicken IgA calibrator.

REAGENT PREPARATION

1. **Diluent Concentrate**

   The Diluent Solution supplied is a 5X Concentrate and must be diluted 1/5 with distilled or deionized water (1 part buffer concentrate, 4 parts dH2O)

2. **Wash Solution Concentrate**

   The Wash Solution supplied is a 20X Concentrate and must be diluted 1/20 with distilled or deionized water (1 part buffer concentrate, 19 parts dH2O). Crystal formation in the concentrate is not uncommon when storage temperatures are low. Warming of the concentrate to 30-35ºC before dilution can dissolve crystals.

3. **Enzyme-Antibody Conjugate**

   Calculate the required amount of working conjugate solution for each microtitre plate test strip by adding 10 µL Enzyme-Antibody Conjugate to 990 µL of 1X Diluent for each test strip to be used for testing. Mix uniformly, but gently. Avoid foaming.

4. **Chromogen Substrate Solution**

   Ready to use as supplied.

5. **Stop Solution**

   Ready to use as supplied.

6. **Anti-Chicken lgA ELISA Micro Plate**

Ready to use as supplied. Unseal Microtiter Pouch and remove plate from pouch. Remove all strips and wells that will not be used in the assay and place back in pouch and re-seal along with desiccant.

7. Chicken IgA Calibrator

Add 1.0 mL of distilled or deionized water to the calibrator and mix gently until dissolved. The calibrator is now at a concentration of 12.133 µg/ml. Chicken IgA standards need to be prepared immediately prior to use (see chart below). Mix well between each step. Avoid foaming.

Standard	Ng/mL	Volume added to 1x Diluent →	Volume of 1x Diluent
6	400	15µL Chicken IgA Calibrator	880µL
5	200	300 µL Standard 6	300 µL
4	100	300 µL standard 5	300 µL
3	50	300 µL standard 4	300 µL
2	25	300 µL Standard 3	300 µL
1	12.5	300 µL Standard 2	300 µL
0	0		600 µL

STORAGE AND STABILITY

1. Diluent Concentrate

The 5X Diluent Concentrate is stable until the expiration date. The 1X working solution is stable for at least one week from the date of preparation. Both solutions should be stored at 4-8ºC.

2. Wash Solution

The 20X Wash Solution Concentrate is stable until the expiration date. The 1X working solution is stable for at least one week from the date of preparation. Both solutions should be stored at 4-8ºC.

3. Enzyme-Antibody Conjugate

Undiluted horseradish peroxidase anti-IgA conjugate should be stored at 4-8ºC and diluted immediately prior to use. The working conjugate solution is stable for up to 1 hour when stored in the dark.

4. Chromogen-Substrate Solution

The Substrate Solution should be stored at 4-8ºC and is stable until the expiration date.

5. Stop Solution

The Stop Solution should be stored at 4-8ºC and is stable until the expiration date.

6. Anti-Chicken IgA ELISA Micro Plate

Anti-Chicken IgA coated wells are stable until the expiration date, and should be stored at 4-8ºC in sealed foil pouch with desiccant pack.

7. **Chicken IgA Calibrator**

   Long Term Storage: Upon receipt, aliquot the calibrator and store them frozen. They will be stable until expiration date. Short Term Storage: the calibrator is stable for up to 14 days at 4ºC. The working standard solutions should be prepared immediately prior to use and are stable for up to 8 hours.

SPECIMEN COLLECTION AND HANDLING

All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions when handling and disposing.

If blood samples are clotted, grossly hemolyzed, lipemic, or the integrity of the sample is of concern, make a note and interpret results with caution.

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

- Serum samples - Blood should be collected by venipuncture. The serum should be separated from the cells after clot formation by centrifugation. Remove serum and assay immediately or aliquot and store samples at -80ºC (preferably) or -20ºC. Avoid repeated freeze-thaw cycles.
- Plasma samples - Blood should be collected into a container with an anticoagulant and then centrifuged. Assay immediately or aliquot and store samples at -80ºC (preferably) or -20ºC. Avoid repeated freeze-thaw cycles.
- Urine samples - Collect mid-stream using sterile or clean urine collector. Centrifuge to remove cell debris. Assay immediately or aliquot and store samples at -80ºC (preferably) or -20ºC. Avoid repeated freeze-thaw cycles.
- Known interfering substances - Azide and thimerosal at concentrations higher than 0.1 % inhibits the enzyme reaction.

DILUTION OF SAMPLES

The assay requires that each test sample be diluted before use. All samples should be assayed in duplicate each time the assay is performed. The recommended dilutions 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. If unsure of sample level, a serial dilution with one or two representative samples before running the entire plate is highly recommended.

- Serum samples - Recommended starting dilution is 1/5,000. To prepare a 1/5,000 dilution of a sample, transfer 5 µL of sample to 495 µL of 1X diluent. This gives you a 1/100 dilution. Next, dilute the 1/100 by transferring 1 0 µL into 490 µL of 1X diluent. This gives you a 1/5,000 dilution. Mix thoroughly each stage.
- Plasma samples - Recommended starting dilution is 1/5,000. To prepare a 1/5,000 dilution of a sample, transfer 5 µL of sample to 495 µL of 1X diluent. This gives you a 1/100 dilution. Next, dilute the 1/100 by transferring 10 µL into 490 µL of 1X diluent. This gives you a 1/5,000 dilution. Mix thoroughly each stage.

MATERIALS REQUIRED BUT NOT PROVIDED

- Precision pipette (2 µL to 200µL) for making and dispensing dilutions
- Test tubes
- Microtitre washer/aspirator
- Distilled or Deionized H2O
- Microtitre Plate reader
- Assorted glassware for the preparation of reagents and buffer solutions
- Timer

ASSAY PROCEDURE

1. **All samples and standards should be assayed in duplicates.**
2. The Standards and the test sample(s) should be loaded into the ELISA wells as quickly as possible to avoid a shift in OD readings. Using a multichannel pipette would reduce this occurrence.

   Pipette 100 µL of

Standard 0	(0.0 ng/ml) in duplicate
Standard 1	(12.50 ng/ml) in duplicate
Standard 2	(25 ng/ml) in duplicate
Standard 3	(50 ng/ml) in duplicate
Standard 4	(100 ng/ml) in duplicate
Standard 5	(200 ng/ml) in duplicate
Standard 6	(400 ng/ml) in duplicate

3. Pipette 100 µL of sample (in duplicate) into pre designated wells.
4. Incubate the micro titer plate at room temperature for twenty (20 ± 2) minutes. Keep plate covered and level during incubation.
5. Following incubation, aspirate the contents of the wells.
6. Completely fill each well with appropriately diluted Wash Solution and aspirate. Repeat three times, for a total of four washes. If washing manually: completely fill wells with wash puffer, invert the plate then pour/shake out the contents in a waste container. Follow this by sharply striking the wells on absorbent paper to remove residual buffer. Repeat 3 times for a total of four washes.
7. Pipette 100 µL of appropriately diluted Enzyme-Antibody Conjugate to each well. Incubate at room temperature for twenty (20 ± 2) minutes. Keep plate covered in the dark and level during incubation.
8. Wash and blot the wells as described in Steps 5/6.
9. Pipette 100 µL of TMB Substrate Solution into each well.
10. Incubate in the dark at room temperature for precisely ten (10) minutes.
11. After ten minutes, add 100 µL of Stop Solution to each well.
12. Determine the absorbance (450 nm) of the contents of each well within 30 minutes. Calibrate the plate reader to manufacturer's specifications.

CALULATION OF RESULTS

1. Subtract the average background value (Average absorbance reading of Standard zero) from the test values for each sample.
2. Average the duplicate readings for each standard and use the results to construct a Standard Curve. Construct the standard curve by reducing the data using computer software capable of generating a four parameter logistic curve fit. A second order polynomial (quadratic) or other curve fits may also be used; however, they will be a less precise fit of the data.
3. Interpolate test sample values from standard curve. Correct for sera dilution factor to arrive at the lgA concentration in original samples.

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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.