Version 06-06/24

User's Manual

Chicken SRBC IgM ELISA Kit

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

This test kit allows rapid and quantitative measurement of chicken anti-SRBC IgM levels in serum or plasma.

General Description

Measurement of anti-SRBC (sheep red blood cell) immunoglobulin levels is used to assess immune function in chickens. To date, a somewhat qualitative hemagglutination assay has often been used to measure antibody titers. Disadvantageously, the hemagglutination assay does not readily differentiate between IgM and IgG (IgY) responses. To address these issues, we at Creative Diagnostics, Inc. have developed an enzyme linked immunosorbant assay (ELISA) that allows rapid and quantitative measurement of chicken anti-SRBC IgM levels in serum or plasma.

Principles of Testing

The chicken anti-SRBC IgM ELISA uses detergent solubilized SRBC ghosts for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated anti-chicken IgM antibodies for detection. Test serum or plasma samples are diluted and incubated in the microtiter wells for 45 minutes. The microtiter wells are subsequently washed, and HRP conjugate is added and incubated for 45 minutes. Anti-SRBC IgM molecules are thus sandwiched between immobilized SRBC antigens and the detection antibody conjugate. The wells are then washed to remove unbound HRP-labeled antibodies, and TMB Reagent is added and incubated for 20 minutes at room temperature. This results in the development of a blue color. Color development is stopped by the addition of Stop Solution, changing the color to yellow, and optical density is measured spectrophotometrically at 450 nm. The concentration of anti-SRBC IgM is proportional to the optical density of the test sample and is derived from a standard curve.

Reagents And Materials Provided

1. SRBC Coated 96-well Plate (provided as 12 strips of 8 wells)
2. Enzyme Conjugate Reagent, 11 mL
3. Reference Standard Stock (lyophilized)
4. 20x Wash Solution, 50 mL
5. Diluent, 30 mL
6. TMB Reagent (One-Step), 11 mL
7. Stop Solution (1N HCl), 11 mL

Materials Required But Not Supplied

1. Precision pipettes and tips
2. Distilled or deionized water
3. Polypropylene or glass tubes

4. Vortex mixer
5. Absorbent paper or paper towels
6. Micro-plate incubator/shaker with mixing speed of ~150 rpm
7. Plate washer
8. Plate reader with an optical density range of 0-4 at 450nm
9. Graph paper (PC graphing software is optional)

Storage

The kit should be stored at 2-8°C and the microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air. Test kits will remain stable for six months from the date of purchase provided that the components are stored as described above.

Specimen Collection And Preparation

General Note: Studies at Creative Diagnostics, Inc. revealed anti-SRBC IgM levels of approximately 10,000 u/mL 7 days after

immunization with SRBC. In order to obtain values within the range of the standard curve, we suggest that samples initially be diluted 500 fold using the following procedure:

1. For each test sample dispense 90 µL and 200 µL of diluent into separate tubes.
2. Pipette and mix 10 µL of the serum/plasma sample into the tube containing 90 µL of diluent. This provides a 10 fold diluted sample.
3. Mix 5 µL of the 10-fold diluted sample with 245 µL of diluent in the second tube to give a 500-fold dilution of the sample.
4. Repeat this procedure for each sample to be tested.

Reagent Preparation

WASH SOLUTION PREPARATION:

The wash solution is provided as a 20x stock. Prior to use, dilute the contents of the bottle (50 mL) with 950 mL of distilled or

deionized water.

STANDARD PREPARATION:

1. Reconstitute the vial of the lyophilized chicken anti-SRBC IgM standard stock with 200 µL of diluents, and then prepare the

100 u/mL standard in a polypropylene or glass tube as described on the vial label.

2. Label 6 polypropylene or glass tubes as 50, 25, 12.5, 6.25, 3.13, and 1.56 u/mL, and dispense 250 µL of diluent into each tube.
3. Prepare a 50 u/mL standard by diluting and mixing 250 µL of the 100 u/mL standard with 250 µL of diluent in the tube labeled 50u/mL.

4. Similarly prepare the 25, 12.5, 6.25, 3.13 and 1.56 u/mL standards by serial dilution.

After reconstitution the standard stock solution is stable for at least one week if stored at 4°C. It should be frozen at or below -20°C if use beyond this time is intended. Avoid multiple freeze-thaws.

Assay Procedure

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 µL of standards and diluted samples into the wells (we recommend that samples and standards be tested in

duplicate).

3. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25C) for 45 minutes.
4. Aspirate the contents of the microtiter wells and wash the wells 5 times with 1x wash solution using a plate washer (400 µL/well). The entire wash procedure should be performed as quickly as possible.
5. Strike the wells sharply onto absorbent paper or paper towels to remove all residual wash buffer.
6. Add 100 µL of enzyme conjugate reagent into each well.
7. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25C) for 45 minutes.
8. Wash as detailed in 4 to 5 above.
9. Dispense 100 µL of TMB Reagent into each well.
10. Gently mix on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25C) for 20 minutes.
11. Stop the reaction by adding 100 µL of Stop Solution to each well.
12. Gently mix. It is important to make sure that all the blue color changes to yellow.
13. Read the optical density at 450 nm with a microtiter plate reader within 5 minutes.

Calculation

1. Calculate the average absorbance values (A450) for each set of reference standards and samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in u/mL on linear graph paper, with absorbance values on the vertical or Y-axis and concentrations on the horizontal or X-axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of anti-SRBC IgM in u/mL from the standard curve.
4. Multiply the derived concentration by the dilution factor to determine the actual concentration of anti-SRBC IgM in the

serum/plasma sample.

5. PC graphing software may be used for the above steps.
6. If the OD450 values of samples fall outside the standard curve when tested at a 500-fold dilution, samples should be diluted

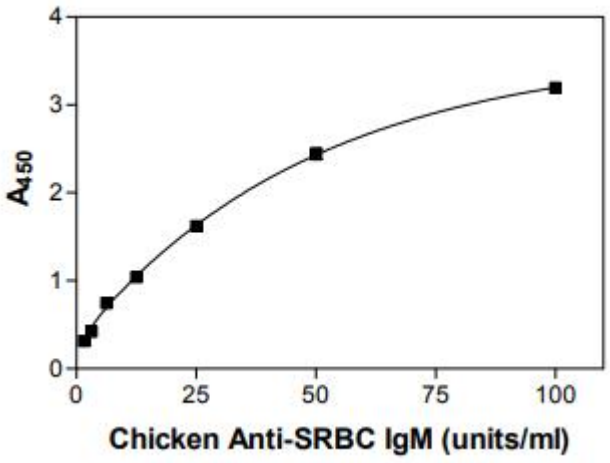
appropriately and re-tested.

Typical Standard Curve

A typical standard curve with optical density readings at 450nm on the Y-axis against anti-SRBC IgM concentrations on the X-axis is

shown below. This curve is for the purpose of illustration only and should not be used to calculate unknowns. Each user should obtain his or her data and standard curve in each experiment.

Anti-SRBC IgM (u/ml)	A_{450}
100	3.196
50	2.440
25	1.620
12.5	1.042
6.25	0.744
3.13	0.421
1.56	0.316



Precautions

1. Please read and understand the instructions thoroughly before using the kit.
2. All reagents should be allowed to reach room temperature (18-25°C) before use.
3. The assay was designed for use with serum or plasma obtained from chickens 5-7 days after immunization with SRBC.
4. The optimal sample dilution should be determined empirically. However, studies performed at Creative Diagnostics, Inc. suggest an initial sample dilution of 500 fold.
5. Optimal results are achieved if, at each step, reagents are pipetted into the wells of the microtiter plate within 5 minutes.

Limitations

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete

understanding

of and in accordance with the instructions detailed above.

2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

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