KAMIYA BIOMEDICAL COMPANY

Turkey IgM ELISA

For the quantitative determination of IgM in turkey serum, plasma and egg-yolk extracts

Cat. No. KT-620

For Research Use Only.

PRODUCT INFORMATION

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PRODUCT

The **K-ASSAY®** Turkey IgM ELISA is an enzyme immunoassay for the quantitative determination of IgM in turkey serum, plasma and egg-yolk extracts. For research use only.

INTRODUCTION

The turkey IgM ELISA kit is intended for measurement of IgM in serum, plasma and egg-yolk extracts. Goat anti-turkey IgM (mu specific chain) antibodies are used for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated goat anti-turkey IgM (mu chain specific) antibodies are used for detection. Normal turkey IgM levels are in the range of 2-5 mg/mL in serum or plasma.

PRINCIPLE

Test samples are diluted and incubated in the microtiter wells for 45 minutes alongside prepared turkey IgM calibrators. The microtiter wells are subsequently washed and HRP conjugate is added and incubated for 45 minutes. IgM molecules are thus sandwiched between the immobilization and detection antibodies. 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 IgM is proportional to the optical density of the test sample and is derived from a calibration curve.

COMPONENTS

- Anti turkey IgM coated 96-well plate (12 strips of 8 wells)
- Reference calibrator (lyophilized)
- 10X Diluent, 25 mL
- HRP Conjugate Reagent, 11 mL
- 20X Wash Solution, 50 mL
- TMB Reagent (One-step), 11 mL
- Stop Solution (1N HCl), 11 mL

MATERIALS REQUIRED BUT NOT PROVIDED

- Precision pipettes and tips
- Distilled or de-ionized water
- Vortex mixer
- Absorbent paper or paper towels
- Graph paper (PC graphing software is optional)
- Polypropylene or glass tubes
- Plate reader with an optical density range of 0-4 at 450 nm.
- Micro-Plate incubator/shaker mixing speed of ~150 rpm
- Plate washer

DILUENT PREPARATION

The diluent is provided as a 10X stock. Prior to use estimate the final volume of diluent required for your assay and dilute one (1) volume of the 10x stock with nine (9) volumes of distilled or de-ionized water.

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

de-ionized water.

CALIBRATOR PREPARATION
1. The IgM calibrator is provided as a lyophilized stock. Reconstitute with 1.0 mL of distilled or de-ionized water *(the reconstituted calibrator is stable at 4°C for one day but should be aliquoted and frozen at -20°C after reconstitution if future use is intended).*
2. Label 7 polypropylene or glass tubes as 200, 100, 50, 25, 12.5, 6.25 and 3.13 ng/mL.
3. Into the tube labeled 200 ng/mL, pipette the volume of diluent detailed on the IgM calibrator vial label. Then add the indicated volume of IgM calibrator and mix gently. This provides the 200 ng/mL calibrator.
4. Dispense 250 µL of diluent into the tubes labeled 100, 50, 25, 12.5, 6.25 and 3.13 ng/mL.
5. Prepare a 100 ng/mL calibrator by diluting and mixing 250 µL of the 200 ng/mL calibrator with 250 µL of diluent in the tube labeled 100 ng/mL.
6. Similarly prepare the 50, 25, 12.5, 6.25 and 3.13 ng/mL calibrators by serial dilution.

SAMPLE PREPARATION
General Note: In order to obtain values within range of the calibration curve, we suggest that samples initially be diluted 40,000 fold using the following procedure for each sample to be tested:

1. Dispense 497.5 µL of 1X diluent into two tubes.
2. Pipette and mix 2.5 µL of the serum/plasma sample into the first tube and mix. This provides a 200 fold diluted sample.
3. Mix 2.5 µL of the 200 fold diluted sample with the 497.5 µL of diluent in the second tube. This provides a 40,000 fold dilution of the sample.
4. Repeat this procedure for each sample to be tested.

ASSAY PROCEDURE
1. Secure the desired number of coated wells in the holder.
2. Dispense 100 µL of calibrators and diluted samples into the wells (we recommend that samples be tested in duplicate).
3. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) 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 (18-25°C) 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 (18-25°C) 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 <u>within 5 minutes</u>.

CALCULATION OF RESULTS
1. Calculate the average absorbance values (A_{450}) for each set of reference calibrators and samples.
2. Construct a calibration curve by plotting the mean absorbance obtained from each reference calibrator against its concentration in ng/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 IgM in ng/mL from the calibration curve.
4. Multiply the derived concentrations by the dilution factor to determine the actual concentration of IgM in the sample.
5. PC graphing software may be used for the above steps.
6. If the OD_{450} values of the sample fall outside the calibration curve, samples should be diluted appropriately and re-tested.

TYPICAL CALIBRATION CURVE
A typical calibration curve with optical density readings at 450 nm on the Y axis against 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 calibration curve in each experiment.

IgM (ng/mL)	Absorbance (450 nm)
200	3.120
100	2.146
50	1.311
25	0.833
12.5	0.540
6.25	0.432
3.13	0.330

Typical Turkey IgM Calibration Curve
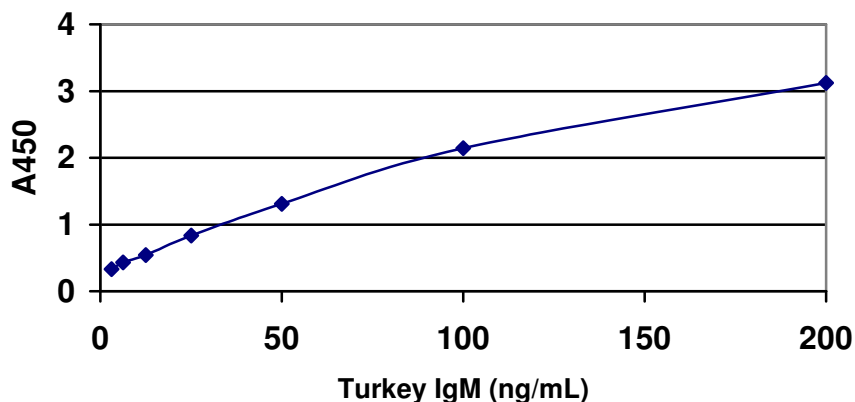
STORAGE

The test kit will remain stable until the expiration date provided that the components are stored as described above. The microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air.

GENERAL INSTRUCTIONS

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. Optimum results are achieved if, at each step, reagents are pipetted into the wells of the microtiter plate within 5 minutes.

LIMITATIONS OF THE PROCEDURE

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.

FOR RESEARCH USE ONLY
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