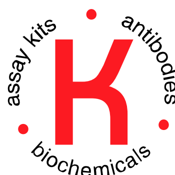

KAMIYA BIOMEDICAL COMPANY

Mouse Anti-Trinitrophenol (TNP) IgG ELISA

For the quantitative determination of anti-TNP IgG in mouse serum and plasma.

Cat. No. KT-692

For Research Use Only.

PRODUCT INFORMATION

Mouse Anti-Trinitrophenol (TNP) IgG ELISA
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PRODUCT
The ***K-ASSAY***® Mouse Anti-Trinitrophenol (TNP) IgG ELISA is an enzyme immunoassay for the quantitative determination of anti-TNP IgG in mouse serum and plasma. For research use only.

INTRODUCTION
Haptens such as trinitrophenol (TNP), when attached to carrier proteins such as ovalbumin or keyhole limpet hemocyanin (KLH) and injected into animals, produce a strong immune response. By measuring changes in the levels of anti-TNP IgM and IgG in appropriate animal models, researchers can assess the impact of pharmacologic or genetic manipulations on the immune system.

PRINCIPLE
The ***K-ASSAY***® Mouse anti-TNP IgG ELISA is based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay uses TNP-BSA as the capture antigen (coated on the microtiter wells) and horseradish peroxidase (HRP) conjugated anti-mouse IgG antibodies for detection. Serum or plasma samples are diluted and incubated alongside calibrators in the microtiter wells for 1 hour. The wells are subsequently washed, and HRP conjugate is added and incubated for 45 minutes. Anti-TNP IgG molecules are thus sandwiched between immobilized TNP 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-TNP IgG is proportional to the absorbance at 450 nm and is derived from a calibration curve.

COMPONENTS
- TNP-BSA Coated Plate (12 x 8 wells)
- Anti-Mouse IgG HRP Conjugate, 11 mL
- Reference Calibrator (lyophilized)
- Diluent, 50 mL
- Wash Solution (20X), 50 mL
- TMB Reagent, 11 mL
- Stop Solution, 11 mL

MATERIALS REQUIRED BUT NOT PROVIDED
- Pipettors and tips
- Distilled or de-ionized water
- Polypropylene or glass tubes
- Vortex mixer
- Absorbent paper or paper towels
- Plate incubator/shaker
- Plate reader capable of measuring absorbance at 450 nm
- Curve fitting software
- Plate washer

GENERAL INSTRUCTIONS
1. All reagents should be allowed to reach 25°C before use.
2. Please read and understand the instructions thoroughly before using the kit.
3. This kit is designed to measure anti-TNP IgG levels in serum collected >14 days after immunization with TNP-carrier protein conjugates. Serum collected at post-immunization times less than 14 days may contain high levels of anti-TNP IgM that compete with anti-TNP IgG for the immobilized TNP, thereby causing interference.
4. Because TNP-modified BSA is used as the capture antigen, it is important that a carrier protein other than albumin (e.g.

KLH or ovalbumin) be used to immunize mice.
5. The optimal sample dilution should be determined empirically. However, our studies suggest an initial sample dilution of 40,000-fold be used. To avoid matrix effects, please do not use dilutions less than 1,000-fold.
6. Optimal results are achieved if, at each step, reagents are pipetted into the wells of the microtiter plate within 5 minutes.
7. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
8. Kits are validated using shaking incubators set at 150 rpm and 25℃. Performance of the assay at lower temperatures and/or mixing speeds will likely result in lower absorbance values.

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. Reconstitute the mouse anti-TNP IgG calibrator as described on the vial label with distilled or de-ionized water. The reconstituted calibrator remains stable at 4℃ for at least one week but should be aliquoted and frozen at -20℃ if use beyond this time is intended.
2. Label 6 polypropylene or glass tubes as 100, 50, 25, 12.5, 6.25 and 3.125 u/mL.
3. In the tube labeled 100 u/mL prepare the 100 u/mL calibrator by diluting 40 µL of reconstituted calibrator with 460 µL of diluent.
4. Dispense 250 µL of diluent into the remaining tubes.
5. Prepare a 50 u/mL calibrator by diluting and mixing 250 µL of the 100 u/mL calibrator with 250 µL of diluent in the tube labeled 50 u/mL.
6. Similarly prepare the 25, 12.5, 6.25 and 3.125 u/mL calibrators by serial dilution.

SAMPLE PREPARATION

In our studies using serum from TNP-KLH immunized mice, levels of 1,562,544 ± 707,478 u/mL (mean ± SD, n = 10) were observed 25 days after immunization. Levels will vary with the immunization protocol and the TNP carrier protein used. We suggest that samples initially be diluted 40,000-fold using the following procedure for each sample to be tested but optimal dilutions must be determined empirically. A 40,000-fold dilution may be achieved as follows:

1. Dispense 249 µL and 318 µL of diluent into separate polypropylene or glass tubes.
2. Pipette and mix 1 µL of the serum sample into the tube containing 249 µL of diluent. This provides a 250-fold diluted sample.
3. Dilute 2 µL of the 250-fold diluted sample into the tube containing 318 µL of diluent and mix. This provides a 40,000-fold dilution.

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 triplicate).
3. Incubate on a plate incubator/shaker at 25℃/150 rpm for 1 hour.
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).
5. Strike the wells sharply onto adsorbent paper or paper towels to remove all residual wash solution.
6. Add 100 µL of HRP conjugate into each well.
7. Incubate on a plate incubator/shaker at 25℃/150 rpm for 45 minutes.
8. Wash as detailed above.
9. Dispense 100 µL of TMB into each well.
10. Incubate on a plate incubator/shaker at 25℃/150 rpm 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 within 5 minutes.

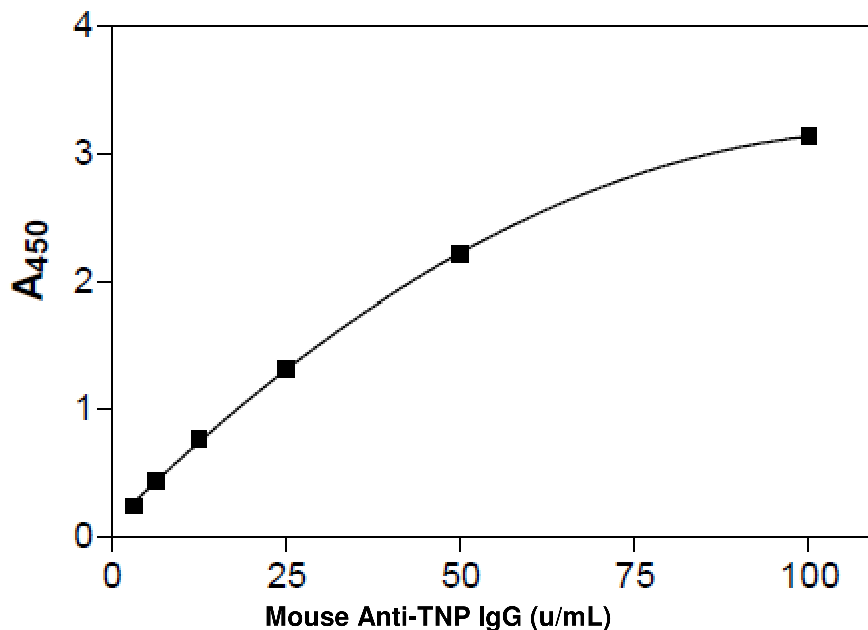
CALCULATION OF RESULTS

1. Using curve fitting software, construct a calibration curve by plotting absorbance values of the calibrators versus concentration.
2. Fit the calibration curve to a second order polynomial model and determine the concentration of the samples from the calibration curve.
3. Multiply the derived concentration by the dilution factor to determine the actual concentration in the samples.
4. If the A_{450} values of samples 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 anti-TNP IgG 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.

Anti-TNP IgG (u/mL)	A_{450}
100	3.141
50	2.210
25	1.318
12.5	0.773
6.25	0.436
3.125	0.243



STORAGE

The kit should be stored at 4°C. The microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air. Test kits will remain stable until the expiration date provided that the components are stored as described above.

FOR RESEARCH USE ONLY

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