Version 24-04/25

User's Manual

Trinitrophenol (Mouse) IgG ELISA Kit

REF **DEIASL079**

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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 is intended for measurement of mouse anti-TNP IgG in serum and plasma samples.

General Description

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 pharamacologic or genetic manipulations on the immune system. Creative Diagnostics, Inc. offers a simple ELISA for measurement of mouse anti-TNP IgG. We also manufacture an ELISA kit for mouse anti-TNP IgM (cat. no. DEIASL080).

Principles of Testing

The mouse anti-TNP IgG test kit is based on a solid phase enzymelinked 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 standards 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 standard curve.

Reagents And Materials Provided

1. TNP-BSA Coated Plate (12 x 8 wells)
2. Anti-Mouse IgG HRP Conjugate, 11 mL
3. Reference Stock (lyophilized)
4. 20x Wash: TBS50-20, 50 mL
5. Diluent: YD50-1. 50 mL
6. TMB: TMB11-1, 11 mL
7. Stop Solution: SS11-1, 11 Ml

Materials Required But Not Supplied

1. Pipettors and tips
2. Distilled or deionized water

3. Polypropylene or glass tubes
4. Vortex mixer
5. Absorbent paper or paper towels
6. Plate incubator/shaker
7. Plate washer
8. Plate reader capable of measuring absorbance at 450 nm.
9. Curve fitting software

Storage

The kit should be stored at 2-8°C. 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

In studies at Creative Diagnostics, Inc., 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.

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 mouse anti-TNP IgG stock as described on the vial label. The reconstituted stock remains stable at 2-8°C for at least one week but should be aliquoted and frozen at -20°C 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 standard as detailed on the stock vial label.
4. Dispense 250 µL of diluent into the remaining tubes.
5. 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 50 u/mL.

6. Similarly prepare the 25, 12.5, 6.25 and 3.125 u/mL standards by serial dilution.

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 be tested in triplicate).
3. Incubate on a plate incubator/shaker at 25°C/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 absorbent 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°C/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°C/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 with within 5 minutes

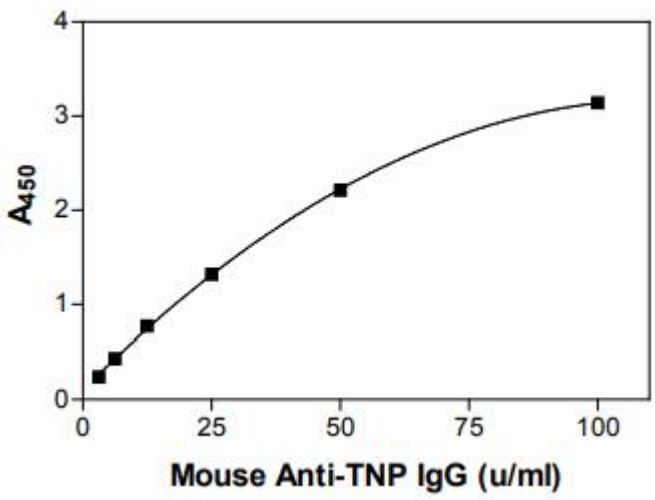
Calculation

1. Using curve fitting software, construct a standard curve by plotting absorbance values of the standards versus concentration.
2. Fit the standard curve to a second order polynomial model and determine the concentration of the samples from the standard curve.
3. Multiply the derived concentration by the dilution factor to determine the actual concentration in the samples.
4. If the A450 values of samples fall outside the standard curve, 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-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



Precautions

1. Please read and understand the instructions thoroughly before using the kit.
2. 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.
3. 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.
4. All reagents should be allowed to reach 25°C before use.
5. The optimal sample dilution should be determined empirically. However, studies performed at Creative Diagnostics, Inc. suggest an initial sample dilution of 40,000-fold be used. To avoid matrix effects, please do not use dilutions less than 1000-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°C. Performance of the assay at lower temperatures and/or mixing speeds will likely result in lower absorbance values.

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.