

# Mouse Anti-TNP IgM ELISA

## Life Diagnostics, Inc., Catalog Number: TNPM-1

### 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. Life Diagnostics, Inc. offers a simple ELISA for measurement of mouse anti-TNP IgM. We also manufacture an ELISA kit for mouse anti-TNP IgG (cat. no. TNPG-1).

### PRINCIPLE OF THE ASSAY

The mouse anti-TNP IgM test kit 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 IgM 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 IgM 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 IgM is proportional to the absorbance at 450 nm and is derived from a standard curve.

### MATERIALS AND COMPONENTS

#### *Materials provided with the kit:*

- TNP-BSA Coated Plate (12 x 8 wells)
- Anti-Mouse IgM HRP Conjugate, 11 ml
- Reference Stock<sup>1</sup> (lyophilized)
- 20x Wash: TBS50-20, 50 ml
- Diluent: YD50-1, 50 ml
- TMB: TMB11-1, 11 ml
- Stop Solution: SS11-1, 11 ml

#### *Materials required but not provided:*

- Pipettors and tips
- Distilled or deionized water
- Polypropylene or glass tubes
- Vortex mixer
- Absorbent paper or paper towels
- Plate incubator/shaker
- Plate washer
- Plate reader capable of measuring absorbance at 450 nm.
- 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.

### GENERAL INSTRUCTIONS/PRECAUTIONS

1. Please read and understand the instructions thoroughly before using the kit.
2. This kit is designed to measure anti-TNP IgM levels in serum collected ~7 days after immunization with TNP-carrier protein conjugates. Serum collected at post-immunization times greater than 7 days may contain high levels of anti-TNP IgG that compete with anti-TNP IgM 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 Life Diagnostics, Inc. suggest an initial sample dilution of 5,000-fold be used. To avoid matrix effects, please do not use dilutions less than 100-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.

### 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. stock. Reconstitute the mouse anti-TNP IgM 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.

<sup>1</sup> Mouse anti-TNP IgM levels are measured in nominal units and are calibrated using pooled anti-TNP mouse serum prepared at Life Diagnostics, Inc.

## SAMPLE PREPARATION

General Note: In studies at Life Diagnostics, Inc., using serum from TNP-KLH immunized mice, levels of  $117,800 \pm 53,432$  u/ml (mean  $\pm$  SD, n = 10) were observed seven days after immunization. Levels will vary with the immunization protocol and the TNP carrier protein. We suggest that samples initially be diluted 5,000-fold using the following procedure for each sample to be tested but optimal dilutions must be determined empirically. A 5,000-fold dilution may be achieved as follows:

1. Dispense 245  $\mu$ l and 297  $\mu$ l of diluent into separate polypropylene or glass tubes.
2. Pipette and mix 5  $\mu$ l of the serum sample into the tube containing 245  $\mu$ l of diluent. This provides a 50-fold diluted sample.
3. Dilute 3  $\mu$ l of the 50-fold diluted sample into the tube containing 297  $\mu$ l of diluent and mix. This provides a 5,000-fold diluted sample.

## ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100  $\mu$ l 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  $\mu$ l/well).
5. Strike the wells sharply onto absorbent paper or paper towels to remove all residual wash solution.
6. Add 100  $\mu$ l 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  $\mu$ l 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  $\mu$ l 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.

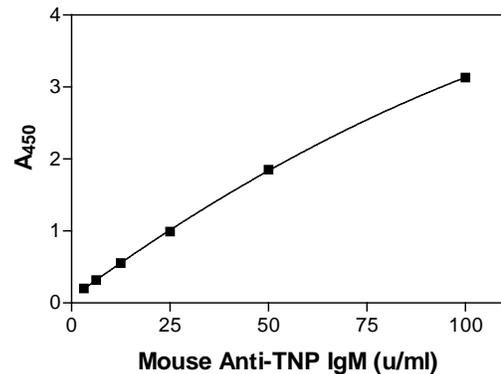
## CALCULATION OF RESULTS

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  $A_{450}$  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 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.

Anti-TNP IgM (u/ml)	$A_{450}$
100	3.132
50	1.851
25	0.991
12.5	0.556
6.25	0.319
3.125	0.201



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For technical assistance please email us at [techsupport@lifediagnostics.com](mailto:techsupport@lifediagnostics.com)