IgM and rat anti-DNP IgM and IgG.

measuring changes in the levels of anti-DNP IgM and IgG in

Color development is stopped by the addition of Stop Solution,

appropriately animal models, researchers can assess the impact of

The mouse anti-DNP IgG test kit is based on a solid phase enzyme-

longitudinally standards in the microtiter wells for 45 minutes. The wells

The wash solution is provided as a 20x stock. Prior to use, dilute the

The kit should be stored at 4°C. The microtiter plate should be kept

The mouse anti-DNP IgG standard is provided as a lyophilized

1 Mouse anti-DNP IgG levels are measured in nominal units and are calibrated using

pooled anti-DNP mouse serum prepared at Life Diagnostics, Inc.
2. Pipette and mix 2 µl of the serum sample into the tube containing 998 µl of diluent. This provides a 500-fold diluted sample.
3. Dilute 2.5 µl of the 500-fold diluted sample into the tube containing 497.5 µl of diluent and mix. This provides a 100,000-fold diluted 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 standards and diluted samples into the wells (we recommend that samples be tested in triplicate).
3. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (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 diluted HRP conjugate into each well.
7. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (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 (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 within 5 minutes.

**CALCULATION OF RESULTS**
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 µ/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-DNP IgG in µ/ml from the standard curve.
4. Multiply the derived concentration by the dilution factor to determine the actual concentration of anti-DNP IgG 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 dilution of 100,000, 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-DNP 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.

<table>
<thead>
<tr>
<th>Anti-DNP IgG (µ/ml)</th>
<th>A450</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>2.844</td>
</tr>
<tr>
<td>50</td>
<td>1.897</td>
</tr>
<tr>
<td>25</td>
<td>1.073</td>
</tr>
<tr>
<td>12.5</td>
<td>0.580</td>
</tr>
<tr>
<td>6.25</td>
<td>0.329</td>
</tr>
<tr>
<td>3.125</td>
<td>0.187</td>
</tr>
</tbody>
</table>

**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.
3. 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.

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For technical assistance please email us at techsupport@lifediagnostics.com