Version 22-04/24

User's Manual

Rabbit Anti-PEG IgM ELISA

REF **DEIA-JY2312**

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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

Principles of Testing

The assay uses immobilized mono mPEGylated BSA (20 kDa PEG chain) as the capture antigen (coated on microtiter wells) and horseradish peroxidase (HRP) conjugated anti-rabbit IgM for detection. Serum or plasma samples are diluted and incubated alongside standards in the microtiter wells for 45 minutes. The wells are subsequently washed, and HRP conjugate is added and incubated for 45 minutes. Anti-PEG IgM molecules are sandwiched between immobilized PEG and the detection antibody conjugate. The wells are then washed to remove unbound HRP-labeled antibodies. 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. Optical density is measured spectrophotometrically at 450 nm. The concentration of anti-PEG IgM is proportional to the absorbance at 450 nm and is derived from a standard curve.

This assay primarily detects antibodies directed against the polyoxyethylene backbone of PEG.

Reagents And Materials Provided

1. PEG-BSA coated plate (12 × 8 - wells) **Store at -20°C.**
2. Anti-IgM HRP Stock: **Store at -20°C.**
3. Anti-PEG Stock: (lyophilized) **Store at -20°C.**
4. 20× HRP PEG Wash: 50 ml.
5. HRP PEG Diluent: 2 × 50 ml.
6. TMB: 11 ml.
7. Stop Solution: 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 reference stock, HRP conjugate, and the PEG-BSA coated plate should be stored at - 20°C. All remaining kit components should be stored at 4°C. The microtiter plate should be kept in a sealed bag with

desiccant. Kits will remain stable for six months from the date of purchase provided that the components are stored as described.

Reagent Preparation

WASH SOLUTION PREPARATION

The wash solution is provided as a 20× stock. Prior to use, dilute the contents of the bottle (50 ml) with 950 ml of distilled or deionized water.

STANDARD PREPARATION

1. The anti-PEG IgM standard is provided as a lyophilized stock. Reconstitute as described on the vial label to obtain the 100 U/ml standard.
2. Label 7 polypropylene or glass tubes as 50, 25, 12.5, 6.25, 3.13, 1.563 and 0 U/ml.
3. Dispense 250 µl of diluent into the tubes.
4. 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.
5. Similarly prepare the 25, 12.5, 6.25, 3.125 and 1.563 u/ml standards by serial dilution.

Unused reconstituted stock should be stored frozen at or below -20°C if future use is intended.

HRP CONJUGATE PREPARATION

Approximately 5 minutes before needed, dilute the HRP conjugate stock with diluent (equilibrated to room temperature) as directed on the vial label.

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 testing in duplicate).
3. Incubate on a plate shaker at 150 rpm/25°C for 45 minutes.
4. Aspirate the contents of the microtiter wells and wash the wells five times with 1x wash solution using a plate washer (400 µl/well).
5. Strike the wells sharply onto absorbent paper to remove all residual wash solution.
6. Add 100 µl of diluted HRP conjugate into each well.
7. Incubate on a plate shaker at 150 rpm/25°C for 45-minutes.
8. Wash as detailed above.
9. Dispense 100 µl of TMB into each well.
10. Incubate on a plate shaker at 150 rpm/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 five minutes.

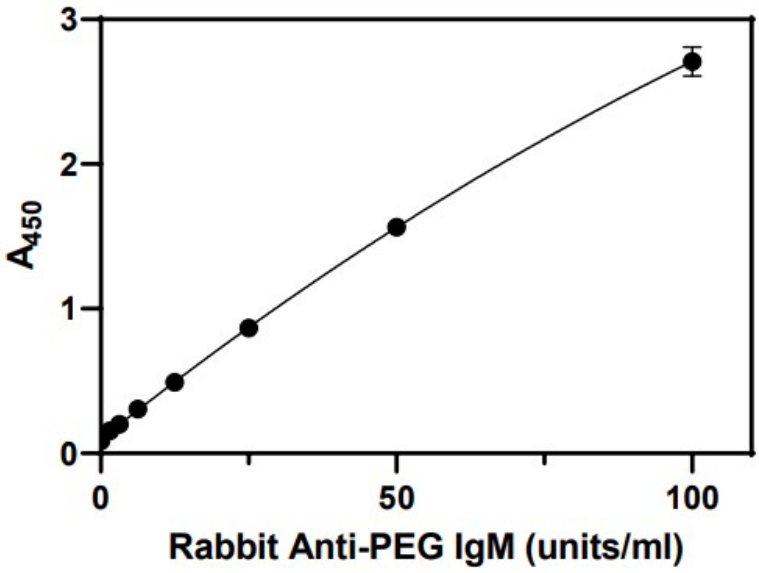
Calculation

1. Using curve fitting software, construct a standard curve by plotting absorbance values of the standards versus concentration.
2. Fit the standard curve to an appropriate model (we fit to two-site, total and non-specific binding model) and determine concentration of the diluted samples from the standard curve.
3. Multiply the derived concentration by the dilution factor to determine concentration in the original samples.
4. If the A450 values of samples fall outside the standard curve, samples should be diluted appropriately and re-tested.

Typical Standard Curve

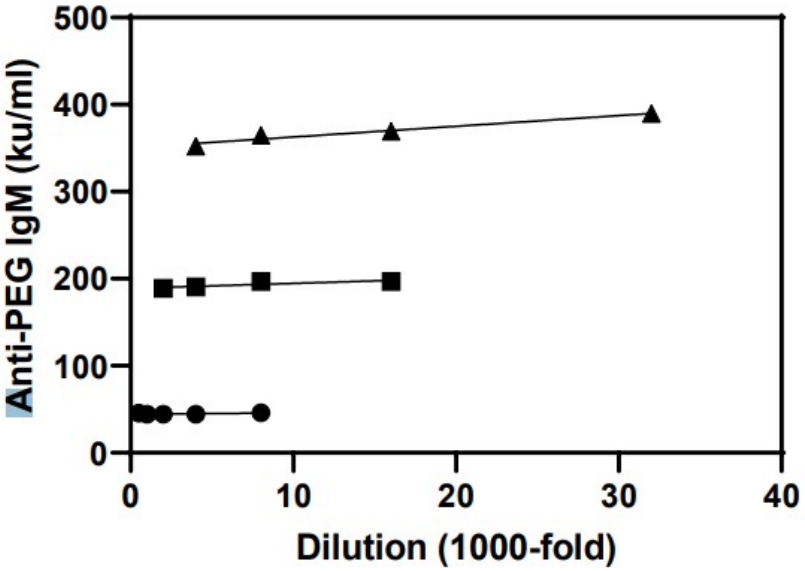
A typical standard curve with optical density readings at 450nm on the Y-axis against anti-PEG IgM concentrations on the X-axis is shown below. This curve is for the purpose of illustration only and should not be used to calculate unknowns.

Anti-PEG IgM (u/ml)	A_{450}
100	2.709
50	1.563
25	0.866
12.5	0.492
6.25	0.305
3.125	0.201
1.563	0.155
0	0.086



Performance Characteristics

Parallelism: To assess performance of the assay, three samples containing anti-PEG IgM at concentrations of 44,886, 193,233 and 368,990 U/ml were serially diluted to produce values within the dynamic range of the assay.



Precautions

1. Please read and instructions thoroughly before using the kit.
2. All reagents should be allowed to reach room temperature (25°C) before use.
3. The wash procedure is critical. Insufficient washing will result inpoor precision and falsely elevated absorbance readings.
4. Use only the wash solution and dilution buffer provided with the kit. PEG and PEGylated compounds are found in many buffers conventionally used in ELISA's and cannot be used with this kit.
5. Kits are validated using plate shakers 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.
6. Optimal results are achieved if at each step reagents are pipetted into the wells of the microtiter plate within 5 minutes.