Version 15-04/25

User's Manual

Human Anti-PEG IgG ELISA Kit

REF **DEIASL243**

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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.

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PRODUCT INFORMATION

Intended Use

This kit is for research use only. Under no circumstances should it be used for therapeutic or human diagnostic applications.

General Description

Attachment of polyethylene glycol (PEG) chains to therapeutic biologic agents, a process referred to as PEGylation, prolongs the circulating half-life of the modified protein by slowing proteolytic degradation and by masking it from the immune system. However, it has been reported that repeated injections of PEGylated proteins can induce anti-PEG antibodies that increase the rate of clearance and decrease drug efficacy (accelerated blood clearance, or ABC phenomenon). To aid research in this key area, we have developed a human anti-PEG IgG ELISA kit.

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-human/monkey IgG monoclonal antibody for detection. Serum and plasma samples are diluted and incubated alongside standards in the microtiter wells for 1 hour. The wells are subsequently washed. HRP conjugate is added and incubated for 45 minutes. Anti-PEG IgG molecules are thus 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 IgG is proportional to the absorbance at 450 nm and is derived from a standard curve.

Reagents And Materials Provided

PEG-BSA coated plate (12 x 8-wells) Store at -20°C

Anti-IgG HRP Stock (lyophilized) Store at -20°C

Reference Stock (lyophilized) Store at -20°C

20x HRP PEG Wash:50 ml

HRP PEG Diluent:50 ml

TMB:11 ml

Stop Solution:11 ml

Materials Required But Not Supplied

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 at 450 nm.

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

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.

DILUENT

The diluent is formulated for measurement PEG antibodies. It is supplied ready to use. DO NOT substitute other buffers.

STANDARDS

1. The anti-PEG IgG standard is provided as a lyophilized stock. Reconstitute as described on the vial label.
2. Label 8 polypropylene or glass tubes as 100, 50, 25, 12.5, 6.25, 3.13, 1.56 and 0.78 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 remaining standards by serial dilution.

SAMPLES

In studies at CD, we found that anti-PEG IgG levels in human samples ranged from undetectable to 3750 u/ml. Optimal dilutions must be determined empirically. However, we suggest that samples initially be diluted 20-fold. To avoid matrix effects, do not test dilutions less than 20-fold (i.e., 10-fold).

HRP CONJUGATE

Approximately 15 minutes before needed, reconstitute the lyophilized HRP conjugate as directed on the vial label and mix gently. Dilute as described on the vial label to give the working conjugate solution. The

reconstituted conjugate stock must be stored at -20°C in a sealed vial if future use is intended.

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 1 hour.
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[2].

[2] On certain plate readers the A450 value of the high standard may be out of range. If that occurs, absorbance values for all wells may be read at 405 nm instead. Absorbance values will be lower, but this does not affect results.

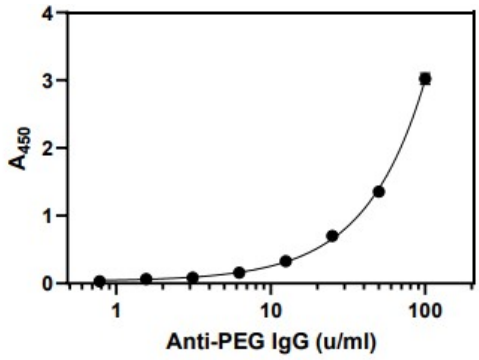
Calculation

1. Using curve fitting software, construct a standard curve by plotting absorbance values of the standards versus log10 of the concentration.
2. Fit the standard curve to a four-parameter logistic regression (4PL) equation (x axis = log10 concentration) and determine the concentration of the samples from the standard curve (remember to derive the antilog).
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

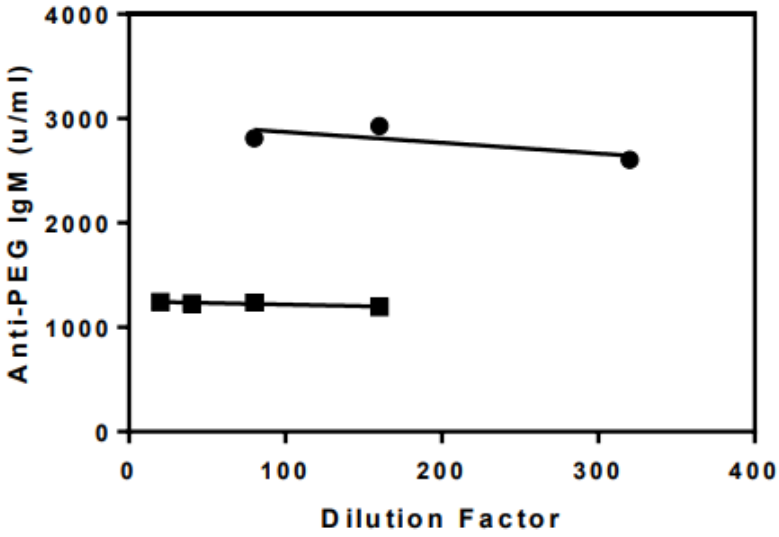
A typical standard curve with optical density readings at 450nm on the Y-axis against anti-PEG IgM concentrations on the X-axis is shown below. This curve is for the purpose of illustration only and should not be used to calculate unknowns.

Anti-PEG IgG (u/ml)	A_{450}
100	3.043
50	1.360
25	0.676
12.5	0.325
6.25	0.155
3.13	0.086
1.56	0.066
0.78	0.051



Performance Characteristics

Parallelism: To assess performance of the assay, two samples sample containing anti-PEG IgG at concentrations of 1226 and 2895 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 in poor 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 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.