Human IgM antibodies to PEG ELISA
Catalog EL-141-PEG-hIGM

For the qualitative determination of anti PEG IgM antibodies in human serum and plasma.

Introduction

Polyethylene glycol (PEG) chains are often used to modify therapeutic biologic agents in order to prolong the circulating half-life of the modified protein by slowing proteolytic degradation. It has been reported that repeat exposure to PEGylated proteins can induce anti-PEG antibodies. Anti-PEG antibodies can result in rapid clearance of PEGylated protein (accelerated blood clearance, or ABC, phenomenon).

Principle of the assay

This immunogenicity assay uses the indirect ELISA technique. Test samples are first centrifuged for 15 minutes to clarify and then diluted using assay buffer. Quality control and test samples are pipetted into the appropriate wells. Anti-PEG antibodies bind the immobilized PEG. After washing, detection antibody (anti-human IgM Peroxidase) is added. Any unbound antibody-enzyme reagent is removed with a final wash and a substrate solution is added to the wells for color development. Color development is proportional to the amount of anti-PEG IgM.

Each kit includes:	Units
Coated microtiter plate, 96 wells (1x8 strips)	1
QC Samples – Serum containing various levels of anti-PEG IgM antibody	QC1 (250µl) anti-PEG 1000ng/ml QC2 (250µl) anti-PEG 500ng/ml QC3 (250µl) anti-PEG 250ng/ml QC4 (250µl) anti-PEG 125ng/ml QC5 (250µl) anti-PEG 62.5ng/ml QC6 (250µl) anti-PEG 0ng/ml
Do not mix or substitute reagents with those from other lots.	

Each kit includes:	Units
1X assay buffer	50ml
10X wash buffer concentrate	50ml
1000X detection reagent	17µl
TMB	12ml
TMB stop solution	12ml
Plate sealers	3
Do not mix or substitute reagents with those from other lots.	

Materials and instruments required but not supplied

- Precision pipettes calibrated to deliver 5-1000µL
- Multi-channel pipette calibrated to deliver 50-200µL
- Plate shaker
- Disposable tips
- Vortex-Mixer
- Distilled or de-ionized water
- Microplate reader capable of reading 450nm with background subtraction at 620nm

Safety precautions

- The test protocol must be followed strictly.
- All reagents containing human material should be handled as if potentially infectious. Operators should wear gloves and protective clothing when handling any patient sera or serum based products.
- The kit reagents contain antimicrobial agents, acid and 3,3',5,5'-tetramethylbenzidine. Avoid contact with the skin and eyes. Rinse immediately with plenty of water if any contact occurs.
- Any liquid that has been brought into contact with potentially infectious material has to be discarded in a container with a disinfectant. Disposal must be performed in accordance with local regulations.
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- Only trained laboratory personnel should execute this test.

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Preparation of reagents

Prepare only the appropriate amount of required reagent on the day of use. Store all reagents as per instructions stated on the label.

1. **Wash Buffer (1X) Preparation:** Dilute wash buffer concentrate with ultra-pure water 1/10 before use (for example add 50mL concentrate to 450mL ultra-pure water). Mix well.
2. **Sample Preparation:** Centrifuge sample for 15 minutes at >15000 X G. Dilute the clarified sample 1 in 5 for assay in the provided assay buffer. For example pipette 50ul of sample into 200µl assay buffer. Mix well.
3. **Detection Reagent (1X) Preparation:** Dilute detection reagent with assay buffer 1/1000 before use (for example add 12µl concentrate to 12ml of assay/wash buffer). Mix well.

Specimen storage

This kit is compatible with EDTA-plasma, heparin-plasma and serum samples. Samples can be stored at or below -20°C for up to 1 year.

Assay procedure

1. Remove kit from -20ºC and allow precoated plate to acclimate to room temperature for 15-20 minutes. Thaw all other components on ice. Add
2. 100µL calibrators and diluted serum test samples to appropriate wells on the plate. Incubate for 1 hour at room temperature on a plate shaker at approximately 300rpm.
3. Discard the content of the plate and wash the wells 3x with 200µL assay/wash buffer per well.
4. Add 100µL detection reagent to all wells. Incubate for 1 hour at room temperature on a plate shaker at approx 300rpm.
5. Discard the content of the plate and wash the wells 3x with 200µL assay/wash buffer per well.
6. Add 100µL of TMB to each well on plate. Incubate for 4-6 minutes at room temperature protected from light.
7. Add 100µL of TMB stop solution to each well on plate. Mix by gently tapping the side of the plate.
8. Determine absorbance with a microplate reader at 450nm against 620nm.

Calculations and results

1. Construct a standard curve by plotting the absorbance obtained from each standard against concentration. Use a 4 or 5 parameter curve fit. Alternatively a log-log curve fit may be used.

   The concentration of the unknowns can be read directly from this standard curve using the absorbance value for each sample.
2. We recommend each lab develop their own statistical cutpoint using methodologies as described by G. Shankar, et al. (2008). (Recommendations for the validation of immunoassays used for detection of host antibodies against biotechnology products. J. Pharmaceutical and Biomedical Analysis 48:1267–1281).
3. Any sample reading greater than the highest standard should be diluted appropriately with assay buffer and retested. If the samples have been diluted, the concentration determined from the standard curve must be multiplied by the dilution factor.

Performance characteristics

Precision: The precision was determined by analyzing samples spiked at 250ng/mL anti-PEG antibody in 6 replicates on 6 different occasions. Intra-assay coefficient of variation (CV) < 10%. Inter-assay CV < 10%.

