Human IgG antibodies to PEG ELISA Catalog EL-141-PEG-hIGG

For the qualitative determination of anti PEG IgG 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. 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. The supplied 96 well microplate is pre-coated with PEG. Quality control and test samples are pipetted into the appropriate wells. Anti-PEG antibodies bind the immobilized PEG. After washing, detection antibody (anti-human IgG 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 IgG.

Each kit includes:	Units
Coated microtiter plate, 96 wells (1x8 strips)	1
QC Samples – Serum containing various levels of anti-PEG IgG antibody **Ready to use - DO NOT DILUTE**	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
10X wash buffer	50ml
Assay buffer	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. **Detection Reagent (1X) Preparation:** Dilute detection reagent with assay buffer 1/1000 before use (for example add 12µl concentrate to 12ml of assay buffer). Mix well.
3. **Sample preparation:** Dilute test samples 1/5 with assay buffer before use (for example add 50µl of test sample to 200µl assay 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.
2. Add 100µL of the pre-diluted calibrators and diluted 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 wash buffer per well.
4. Add 100µL of the prepared 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 wash buffer per well.
6. Add 100µL of TMB to each well on plate. Incubate for 5-8 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 from the standard curve using the absorbance value for each sample and multiplied by the dilution factor.
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%.

Ordering Information

Please vist www.affinityimmuno.com to order this product. Visa, Mastercard, AMEX and PayPal are accepted in our online store.

Your order will be processed immediately and you will be notified with a delivery timeframe.

Materials and storage

Store kit components at -20°C unless specified otherwise. DO NOT USE past kit expiration date. Some vials contain a small amount of reagents. Spin tubes on pulse setting prior to opening.