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ab215546 Polyethylene Glycol (PEG) ELISA Kit

For the competitive quantitative measurement of PEGylated molecules in plasma, serum and cell culture media.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Polyethylene Glycol (PEG) RabMab® *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for accurate competitive quantitative measurement of PEGylated molecules in plasma, serum and cell culture media.

The Polyethylene Glycol (PEG) RabMab® ELISA Kit operates on the basis of competition between enzyme HRP conjugated PEG and PEG labeled molecules for a limited number of binding sites on the surface of 96-wells coated with anti-PEG RabMab® antibody. The extent of color development resulting from interaction between HRP and the substrate TMB is inversely proportional to the number of PEGylated molecules in the sample. For example, the absence of PEGylated molecules in the sample will result in a bright blue color, whereas the presence of PEGylated molecules will result in decreased or no color development.

Polyethylene glycol (PEG) is an O-CH_2-CH_2 polymer, which is water-soluble, nontoxic, non-antigenic, and biocompatible. Covalent conjugation of PEG to therapeutic proteins increases the in vivo stability by protecting the protein from degradation, masking its immunogenic sites and reducing clearance. Typically, PEGylation uses nonspecific reactions with nucleophilic residues and produces mixtures of PEGylated positional isomers. Qualitative and quantitative analysis of PEGylated molecules is important for both drug development and clinical application. This kit is developed to determine levels of PEGylated molecules in samples such as serum, plasma or cell culture medium via ELISA.

Users must PEGylate their interested molecules. For pharmacokinetic experiments, the PEGylated molecules will be used to construct standard curves.

The use of this kit requires the end user to have at least 250 ng of PEGylated compound of interest to use as reference standard. The included PEG-BSA is a reference sample only.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed

Mix 1X PEG-HRP with the standard series, test samples or controls

Add 50 µL/well mixture to appropriate wells

Incubate at room temperature for 45 minutes

Aspirate and wash each well three times with 350 µL 1X Wash Buffer PT

Add 100 µL TMB Substrate to each well and incubate for 15 minutes

Add 100 µL Stop Solution and read OD at 450 nm

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handle with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at +4°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Reagent Preparation section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
PEG conjugated HRP 60X	150 µL	+4°C
Reference Standard (PEG-BSA)	200 ng	+4°C
Antigen/Antibody Diluent Buffer 1X	20 mL	+4°C
Wash Buffer PT 10X	20 mL	+4°C
TMB Substrate	12 mL	+4°C
Stop Solution	12 mL	+4°C
Anti-PEG coated microplate (12 x 8 well strips)	96 Wells	+4°C
Plate Seal	1	+4°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at 450 or 600 nm.
- Method for determining protein concentration (BCA assay recommended).
- Deionized water.
- Multi- and single-channel pipettes.
- Tubes for standard dilution.
- Plate shaker for all incubation steps.
- Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).
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8. Technical Hints

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- As a guide, typical ranges of sample concentration for commonly used sample types are shown below in Sample Preparation (section 11).
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.
- Incubate ELISA plates on a plate shaker during all incubation steps.
- When generating positive control samples, it is advisable to change pipette tips after each step.

- The provided Antigen/Antibody Diluent Buffer contains 0.01% Thimerosal. Additional protease inhibitors can be added if required.
- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.
- Prepare only as much reagent as is needed on the day of the experiment. PEG Conjugated HRP has only been tested for stability in the provided 60X formulation.

9.1 1X PEG Conjugated HRP

Equilibrate PEG 60X conjugated HRP to room temperature before diluting to 1X with Antigen/Antibody Diluent Buffer. Prepare 1X PEG Conjugated HRP by diluting 60X PEG Conjugated HRP with Antigen/Antibody Diluent Buffer. To make 3 mL 1X PEG Conjugated HRP, combine 50 µL 60X PEG conjugated HRP with 2,950 µL Antigen/Antibody Diluent Buffer. Mix thoroughly and gently.

9.2 1X Wash Buffer PT:

Prepare 1X Wash Buffer PT by diluting Wash Buffer PT 10X with deionized water. To make 60 mL 1X Wash Buffer PT combine 6 mL Wash Buffer PT 10X with 54 mL deionized water. Mix thoroughly and gently.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

10.1 PEG - BSA Reference Standard Dilution (provided with the kit as a reference sample)

10.1.1 Label eight tubes, Standards 1– 8

10.1.2 Reconstitute the PEG-BSA standard sample by adding 1 mL distilled water. Hold at room temperature for 10 minutes and mix gently. This is the 200 ng/mL **Stock Standard** Solution. Transfer 325 µL of this Stock standard solution to tube #1 to create PEG-BSA **Standard #1**.

10.1.3 Prepare PEG-BSA **Standard #2** by adding 75 µL Antigen/Antibody Diluent Buffer into tube Standard #2 then transferring 225 µL from PEG-BSA **Standard #1** to tube Standard #2. Mix thoroughly and gently

10.1.4 Prepare PEG-BSA **Standard #3** by adding 100 µL Antigen/Antibody Diluent Buffer into tube Standard #3 then transferring 200 µL from PEG-BSA **Standard #2** to tube Standard #3. Mix thoroughly and gently.

10.1.5 Using the table below as a guide to create PEG-BSA **Standards #4** through **#6**.

10.1.6 PEG-BSA Standards **#7** & **#8** contain no protein and are the Blank controls. For Standard **#8**, the PEG-HRP solutions should be omitted from addition to the wells during Step 12.3.

PEG-BSA Standard #	Sample too Dilute	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (ng/mL)	Final Conc. (ng/mL)
1	See Step 10.1.2				200
2	Standard #1	225	75	200	150
3	Standard #2	200	100	150	100
4	Standard #3	100	100	100	50
5	Standard #4	40	160	50	10
6	Standard #5	40	160	10	2
7 (Blank)	N/A	NA	100	0	0

8 (No HRP blank)	N/A	NA	100	0	0

- For the PEGylated sample of interest, make a series dilution using either a 2, 3 or 4-fold dilution depending on number of data points desired. Start with between 2,000 and 3,000 ng/mL of PEGylated sample and dilute down to about 2 ng/mL.
- Example below with PEG-Mouse-IgG as the PEGylated sample of interest, using a 4-fold dilution from a stock solution at 2,500 ng/mL

10.2 **PEGylated Sample Standard Dilution** (this is an example of user's reference sample, DID NOT provide with the kit)

10.2.1 Label eight tubes, Standards 1– 8.

10.2.2 Reconstitute / dilute PEG-Mouse-IgG with distilled water to produce a stock solution of 2,500 ng/mL. Transfer 200 µL of this stock solution to tube #1 to create PEG-Mouse-IgG **Standard #1**.

10.2.3 Add 150 µL Antigen/Antibody Diluent Buffer into tube #2 – 8.

10.2.4 Prepare PEG-Mouse-IgG **Standard #2** by transferring 50 µL from PEG-Mouse-IgG **Standard #1** to tube #2. Mix thoroughly and gently.

10.2.5 Prepare PEG-Mouse-IgG **Standard #3** by transferring 50 µL from PEG-Mouse-IgG **Standard #2** to tube #3. Mix thoroughly and gently.

10.2.6 Using the table below as a guide, repeat for tubes #4 through #6.

10.2.7 Sample **Standards #7** & **#8** contain no protein and are the Blank controls. For Standard **#8**, the PEG-HRP solutions should be omitted from addition to the wells during Step 12.3.

PEG-Mouse-IgG Standard #	Sample too Dilute	Volume too Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (ng/mL)	Final Conc. (ng/mL)
1	See Step 10.2.2				2,500
2	Standard #1	50	150	2,500	625
3	Standard #2	50	150	625	157
4	Standard #3	50	150	157	39
5	Standard #4	50	150	39	9.8
6	Standard #5	50	150	9.8	2.4

7 (Blank)	N/A	N/A	150	0	0
8 (No HRP Blank)	N/A	N/A	150	0	0

11. Plate Preparation

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.
- For each assay performed, a minimum of two wells must be used as the zero control, which omit PEG-HRP from well additions (such as Standard #8).
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Differences in well absorbance or "edge effects" have not been observed with this assay.

12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.
- Prepare all reagents, working standards, and samples as directed in the previous sections.

12.1 Prepare all reagents, working standards, and samples as directed in the previous sections.

12.2 Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.

12.3 For each well, mix 50 µL of 1X PEG-HRP (see section 9.1) with 50 µL of the standard series (e.g. PEG-BSA, PEG-IgG), test samples or control.

12.4 Add 50 µL of the mixture from 12.3 above to each well.

12.5 Seal the plate and incubate for 45 minutes at room temperature on a plate shaker set to 400 rpm.

12.6 Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and blot it against clean paper towels to remove excess liquid.

12.7 Add 100 µL of TMB Substrate to each well and incubate for 15 minutes in the dark on a plate shaker set to 400 rpm.

12.8 Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.

12.9 Analyze the data as described below.

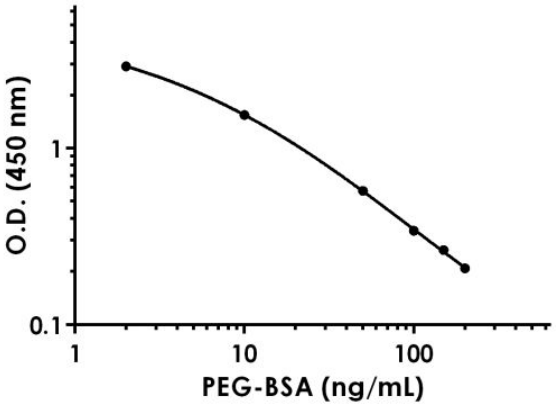
13. Calculations

Calculate the average absorbance values (A450) for each set of standards (or references) and samples. Construct a standard curve by setting concentration of each point in the standard serial of dilution in ng/mL along the X axis and the no PEG-HRP blank control subtracted mean absorbance obtained for each standard point as Y axis. Create a curve for the plotted standard dilution series using a power trend line. The curve will generate the equation: y=Ax-B and also generate a R2 value.

Using the no PEG-HRP blank control subtracted mean absorbance value for each sample, determine the corresponding concentration of sample in ng/mL (X) from the equation: X=10^(log A-log Y)/B)

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only.** A new standard curve must be generated for each assay performed.



Standard Curve Measurements			
Concentration (ng/mL)	O.D 450 nm		Mean O.D
	1	2	
0 (no PEG-HRP)	0.046	0.051	0.049
0	3.391	3.357	3.374
2	2.925	2.914	2.920
10	1.544	1.550	1.547
50	0.578	0.567	0.572
100	0.339	0.342	0.341
150	0.264	0.266	0.265
200	0.205	0.211	0.208

Figure 1. Example of PEG BSA standard curve. The PEG BSA standard curve was prepared as described in Section 10. Raw data values are shown in the table.

RECOVERY

Three concentrations of PEG_BSA were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

Sample Type	Average % Recovery	Range (%)
Antigen/Antibody Diluent Buffer	100	100
50% Human Serum	91	82 - 101
50% Human Plasma - Citrate	79	75 - 85
50% Human Plasma - EDTA	84	77 - 90
50% Human Plasma - Heparin	70	64 - 77
50% Cell Culture Media	104	99 - 108

PRECISION

Mean coefficient of variations of interpolated values from PEG-BSA within the working range of the assay

	Intra-Assay	Inter-Assay
n =	4	4
CV(%)	3%	6%

15. Assay Specificity

Monomethoxy PEG (mPEG), with the molecular weight about 5 kDa, is used to immunize rabbits to generate anti-PEG antibody and modify BSA in our experiments. Rabbit monoclonal RabMab® Anti-PEG specifically recognizes the methoxy group of mPEG.

Please contact our Technical Support team for more information

16. Troubleshooting

Problem	Reason	Solution
Poor standard curve	Inaccurate Pipetting	Check pipettes
	Improper standard dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
	Standard improperly reconstituted	Briefly spin vial before opening; thoroughly resuspend powder
	Standard degraded	Store sample as recommended
	Curve does not fit scale	Try plotting using different scale
Low Signal	Incubation times too brief	Ensure sufficient incubation times: try incubation overnight at 4˚C.
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
	sample prepared incorrectly	Ensure proper sample preparation/dilution
	Using incompatible sample type (e.g. serum vs. cell extract)	Detection may be reduced or absent in untested sample type
	Precipitate can form in wells upon substrate addition when concentration of target is too high	Increase dilution factor of sample
	Target present below detection limits of assay	Decrease dilution factor, or concentrate samples
High background	Wells are insufficiently washed	Wash wells as per protocol recommendations
	Waiting too long to read plate after adding STOP solution	Read plate immediately as per protocol recommendations
	Contaminated wash buffer	Make fresh wash buffer
Large CV	all wells not washed	Review manual for proper wash

	equally/thoroughly	technique. If using a plate washer, check all ports for obstructions.
	Incomplete reagent mixing	Ensure all reagents/master mixes are mixed thoroughly
	Inconsistent pipetting	Use calibrated pipettes and ensure accurate pipetting
	Inconsistent sample preparation or storage	Preparation and optimal sample storage conditions (e.g. minimize freeze/thaws cycles)
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Using incompatible sample type (e.g. serum vs. cell extract)	Detection may be reduced or absent in untested sample type
	Improper storage of the ELISA kit	Store your reconstituted standards at -80°C, all other assay components 4°C. Keep TMB substrate solution protected from light.

17. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)