Product name: Anti-Polyethylene glycol antibody [PEG-2-128] ab133471

Description: Rabbit monoclonal [PEG-2-128] to Polyethylene glycol

Host species: Rabbit

Tested applications: Suitable for: Sandwich ELISA, WB, ELISA

Species reactivity: Reacts with: Species independent

Immunogen: Chemical/ Small Molecule corresponding to Polyethylene glycol conjugated to keyhole limpet haemocyanin. Synthetic peptide corresponds to Polyethylene glycol 20K (PEG20K).

Epitope: This antibody recognizes the backbone of the Polyethylene glycol molecule. It can therefore be used to detect free forms of PEG. To detect conjugated forms of PEG, please see ab51257, which detects the terminal methoxy group of the PEG molecule.

General notes: Antibody Isotype: Please note, this RabMAb product's isotype is Rabbit IgM.

As this product's isotype is Rabbit IgM, KD values were provided by loading the antigen first before testing the antibody.

When compared to loading the antibody first and then the antigen, this method can give approximately 10-1 difference as determined by using ab51257 - Anti-Polyethylene glycol antibody [PEG-B-47] (of rabbit IgG isotype) as a control:

Antigen loaded first: 1.51E-10. Antibody loaded first: 2.41E-09.

Tween 20 usage: Tween 20 is a detergent that can interfere with ELISA and potentially other applications (often used in antibody dilution buffers, such as TBST) when using anti-PEG products. This is also the same case for other polyoxyethylene detergents. Therefore, it is recommended to avoid Tween 20 and other polyoxyethylene detergents when performing ELISA using anti-PEG products.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is
designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.

Properties

Form
Liquid

Storage instructions
Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.

Dissociation constant ($K_D$)
$K_D = 8.24 \times 10^{-12} \text{ M}$

Storage buffer
Preservative: 0.01% Sodium azide
Constituents: 50% Tissue culture supernatant, 9% PBS, 50% Glycerol, 0.05% BSA

Purification notes
Harvested from hybridoma, preliminarily purified via H2O dialysis and centrifuged to remove impurities.

Primary antibody notes
ab133471 is useful in measuring the pharmacokinetics of Polyethylene glycol modified molecules.

Clonality
Monoclonal

Clone number
PEG-2-128

Isotype
IgM

Applications

Our Abpromise guarantee covers the use of ab133471 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sandwich ELISA</td>
<td>Use at an assay dependent concentration. 10000 ng/ml.</td>
<td></td>
</tr>
<tr>
<td>WB</td>
<td>1/1000. The MW of the WB band for ab133471 varies based on the PEGylated molecule detected.</td>
<td></td>
</tr>
<tr>
<td>ELISA</td>
<td>Use a concentration of 2 - 10 µg/ml.</td>
<td></td>
</tr>
</tbody>
</table>

Target

Images
Direct ELISA antigen dose-response curve using purified ab133471. Antigen concentration of 0-10000 ng/mL. A HRP-conjugated goat anti-rabbit IgM (1/1000) was used as the secondary antibody.

Sandwich ELISA antigen dose-response curve using purified ab133471.
Capture antibody - ab133471 (10000 ng/ml).
Antigens - MBP-PEG20K and ADI-PEG (0-1000 ng/ml).
Detection - ab53449 (500 ng) anti-Polyethylene glycol antibody [PEG-B-47b] (Biotin) followed by peroxidase-conjugated streptavidin (1/1000).

Direct ELISA antigen dose-response curve using purified ab133471. Antigen concentration of 0-100 ng/mL. A HRP-conjugated goat anti-rabbit IgM (1/1000) was used as the secondary antibody.
**Western blot - Anti-Polyethylene glycol antibody [PEG-2-128] (ab133471)**

**All lanes**: Anti-Polyethylene glycol antibody [PEG-2-128] (ab133471) at 1/1000 dilution (purified)

**Lane 1**: PEG20K

**Lane 2**: MBP-PEG20K

Lysates/proteins at 5 µg per lane.

**Secondary**

**All lanes**: Peroxidase-conjugated goat anti-rabbit IgM, (H+L) at 1/500 dilution

Blocking buffer: 15% BSA/TBS.

Dilution buffer: 10% PAA/TBST.

**Western blot - Anti-Polyethylene glycol antibody [PEG-2-128] (ab133471)**

**All lanes**: Anti-Polyethylene glycol antibody [PEG-2-128] (ab133471) at 1/1000 dilution (unpurified)

**Lane 1**: MBP-PEG20K at 1 µg

**Lane 2**: MBP-PEG20K at 0.5 µg

**Lane 3**: PEG20K at 1 µg

**Lane 4**: PEG20K at 0.5 µg

**Secondary**

**All lanes**: HRP-conjugated goat anti-rabbit IgM at 1/2000 dilution

Gel concentration: 4-20%

**ELISA - Anti-Polyethylene glycol antibody [PEG-2-128] (ab133471)**

Various concentrations of Polyethylene glycol polymers with amine end groups were coated in 96 wells. 2 µg/ml of unpurified ab133471 was added. After washing, samples were incubated with 1 µg/ml of HRP-conjugated anti-rabbit IgM.
Various concentrations of Polyethylene glycol 20K (PEG20K) conjugated MBP and branched Polyethylene glycol conjugated BSA were coated in 96 wells. 2 ug/ml of unpurified ab133471 was added. After washing, samples were incubated with 1 ug/ml of HRP-conjugated anti-rabbit IgM.

10 ug/ml of unpurified ab133471 was coated into 96 wells. Serial dilution of Polyethylene glycol 20K (PEG20K) conjugated MBP and PEGylated ADI were added. After washing, 1 ug/ml of biotinylated anti-Polyethylene glycol antibody was added. Streptavidin-HRP was then used to develop the color.

Method: APS sensor + Antigen (PEG 5K-BSA at 0.3µg/ml)+ antibody (ab133471 at 0.0312, 0.0625, 0.125, 0.25, 0.5, 1 µg/m). Sample ID: PEG-2-128

KD (M): 8.24E-12
kon(1/Ms): 1.47E+07
kon Error: 3.59E+04
kdis(1/s): 1.22E-04
kdis Error: 6.46E-07
Full X^2: 6.0601
Full R^2: 0.9985

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
• Response to your inquiry within 24 hours
• We provide support in Chinese, English, French, German, Japanese and Spanish
• Extensive multi-media technical resources to help you
• We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit https://www.abcam.com/abpromise or contact our technical team.

Terms and conditions

• Guarantee only valid for products bought direct from Abcam or one of our authorized distributors