Product name: Anti-Polyethylene glycol antibody [PEG-B-47] ab51257

Description: Rabbit monoclonal [PEG-B-47] to Polyethylene glycol

Host species: Rabbit

Specificity: This antibody recognizes the terminal methoxy group of the PEG molecule. This antibody does not cross react with non-specific targets in blood or serum. Both free form of PEG (unconjugated version) and conjugated PEG were tested by sandwich ELISA using ab51257; results showed that ab51257 only detects conjugated forms of PEG. To detect free form of PEG, we recommend using ab133471. ab133471 recognizes PEG backbone and that PEG is not required to be bound to other molecules.

Tested applications: Suitable for: ELISA, WB, IHC-P

Immunogen: Chemical/Small Molecule corresponding to Polyethylene glycol conjugated to keyhole limpet haemocyanin. KLH-Polyethylene glycol with a terminal methoxy group.

General notes: If you have any questions on our PEG products - please visit our Polyethylene glycol (PEG) FAQs page.

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

This product is a recombinant rabbit monoclonal antibody.

Properties:

Form: Liquid

Storage instructions: Shipped at 4°C. Upon delivery aliquot. Store at -20°C.

Dissociation constant (K_D): K_D = 2.41 x 10^{-9} M

Storage buffer: pH: 7.40
Preservative: 0.01% Sodium azide
Constituents: 40% Glycerol, 0.05% BSA, 59% PBS

Purity
Protein A purified

Clonality
Monoclonal

Clone number
PEG-B-47

Isotype
IgG

Applications

Our Abpromise guarantee covers the use of ab51257 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

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<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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<tr>
<td>ELISA</td>
<td>Use a concentration of 0.5 - 5 µg/ml.</td>
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<tr>
<td>WB</td>
<td>Use a concentration of 0.015 - 0.075 µg/ml.</td>
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<tr>
<td>IHC-P</td>
<td>1/100. This antibody only works on the tissues when the animals are injected with a PEGylated protein. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.</td>
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Target

Immunohistochemistry images of anti-PEG (brown) and hematoxylin counterstaining (blue) of tumor (A1–A3 and B1–B3) and muscle tissue (A4 and B4) 30 minutes (A) and 2 h (B) post injection of Spago Pix

Tumor tissue at both time points is shown at three different enlargements where the enlarged areas are indicated by the square boxes. Vessels (V), fibroblasts (Fb) and fat cells (Fc) are indicated with rounded lines. Note that the tumor tissue in panel B1–B3 originates from a mouse injected with a dose of 10 µmol Mn/kg, whereas the other tissues originate from animals administered 20 µmol Mn/kg.

Paraformaldehyde fixed muscle and tumor tissues collected before injection and at 30 minutes and 2–4 h post-dose were paraffin embedded and thereafter cut (4 µm) and positioned on glass tissue slides. Prior to staining, the slides were placed in an antigen-retrieval solution using an automated pre-treatment module (PT-Link; Dako, Glostrup, Denmark). Slides were stained for PEG in an
automated immunohistochemistry robot (Autostainer; Dako). PEG is detected with ab51257 at 0.6 μg/ml.

(From Figure 6 Eriksson et al)

Comparison of sandwich ELISA using RabMAb/RabMAb (ab51257/ab51257) and MAb/RabMAb (Vendor A/ab51257) for capture/detection.

Ab51257/ab51257*: Plate coated with 5 ug/mL of #47; 5 ug/mL of #47 used for detection (*Anti-PEG 47 biotin labeled)

Vendor A/ ab51257*: Plates coated with 100 ug/mL of Vendor A Mouse MAb; 5 ug/mL of #47 used for detection (*Anti-PEG 47 biotin labeled)

Comparison of 10 ug/well of activated linear (PEG5K) and branched (PEG40K) PEG using 5 ug/ml of ab51257 in Direct ELISA assay.

Accuracy: By detecting the methoxy group of the PEG molecule itself, ab51257 is useful in measuring the pharmacokinetics of PEG-modified molecules in vivo. Data indicate that ab51257 detects various length Y-chain PEG molecules as well as single chain PEG molecules with equal affinity. Ab51257 does not cross react with non-specific targets in blood or serum.

Comparison of ab51257 and Vendor A mouse MAb in Direct ELISA assay.

Goat anti-rabbit IgG-AP used for anti-PEG-47 detection; goat anti-mouse IgM-AP used for Vendor A MAb detection.

Fig 1a. Direct ELISA using 1 ug/mL of PEG-GCSF.

Fig 1b. Direct ELISA using 1 ug/mL of PEG-IFN.

Comparison to other anti-PEG: In both direct and sandwich ELISA assays, ab51257 shows greater affinity and accuracy than other anti-PEG antibodies when determining the concentration of PEG or PEG-modified proteins. Results were similar whether detecting PEG itself or PEG-modified targets.
ELISA assay using ab51257 to detect different forms of PEG. PEG40000-BSA is a 40 kDa PEG molecule attached to BSA. PEG5000-BSA is a 5 kDa linear PEG molecule attached to BSA.

Anti-Polyethylene glycol antibody [PEG-B-47] (ab51257) at 0.015 µg/ml + BSA-PEG at 10 µg

**Secondary**
Goat Anti-Rabbit IgG, (H+L), HRP-conjugated at 1/1000 dilution

Blocking buffer and concentration: 5% NFDM/TBST.
Diluting buffer and concentration: 5% NFDM/TBST.

Immunoblot of different antigen-Polyethylene glycol preparations. Western Blot images using ab51257, unpurified, to detect BSA-PEG.
Lane 1: 300 ng/ml;
Lane 2: 75 ng/ml;
Lane 3: 15 ng/ml;
Lane 4: 1.5 ng/ml.
Other - Anti-Polyethylene glycol antibody [PEG-B-47] (ab51257)

Method: Protein A sensor + Antigen (PEG 5K-BSA at 0.3µg/ml) + antibody (ab51257 at 0.0312, 0.0625, 0.125, 0.25, 0.5, 1 µg/ml).

Results:

Sample ID: PEG-5K-BSA

KD (M): 2.41E-09

kon(1/Ms): 2.67E+04

kon Error: 6.81E+02

kdis(1/s): 6.43E-05

kdis Error: 2.92E-06

Full X^2: 0.018444

Full R^2: 0.982059

ab51257, unpurified, staining mouse kidney at 1:10 dilution. Left panel: without animal injection with a PEGylated protein. Right panel: with animal injection with a PEGylated protein.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Polyethylene glycol antibody [PEG-B-47] (ab51257)

ab51257, unpurified, staining mouse muscle at 1:10 dilution. Left panel: without animal injection with a PEGylated protein. Right panel: with animal injection with a PEGylated protein.
ab51257, unpurified, staining mouse liver at 1:10 dilution. Left panel: without animal injection with a PEGylated protein. Right panel: with animal injection with a PEGylated protein.

ab51257, unpurified, staining mouse spleen at 1:10 dilution. Left panel: without animal injection with a PEGylated protein. Right panel: with animal injection with a PEGylated protein.

Sandwich ELISA-Left Graph generated using unpurified ab51257 and right using purified ab51257 at 1μg/mL. Antigen concentration range 0.0156 - 1 μg/mL for PEG-IFN-α-2b. Secondary antibody was an Alkaline Phosphatase-conjugated Goat Anti-Rabbit IgG(H+L) at 1/2500.
Direct ELISA-Left Graph generated using unpurified ab51257 and right using purified ab51257 at 1μg/mL. Antigen concentration range 0.0039 - 0.25μg/mL for PEG-BSA. Secondary antibody was an Alkaline Phosphatase-conjugated Goat Anti-Rabbit IgG(H+L) at 1/2500.

Direct ELISA-Left Graph generated using unpurified ab51257 and right using purified ab51257 at 1μg/mL. Antigen concentration range 0.0156 - 1 μg/mL for ADI-PEG. Secondary antibody was an Alkaline Phosphatase-conjugated Goat Anti-Rabbit IgG(H+L) at 1/2500.

Direct ELISA-Left Graph generated using unpurified ab51257 and right using purified ab51257 at 1μg/mL. Antigen concentration range 0.0078 - 0.5 μg/mL for both PEG5000 and PEG20000. Secondary antibody was an Alkaline Phosphatase-conjugated Goat Anti-Rabbit IgG(H+L) at 1/2500.

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

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