# abcam

### Product datasheet

## Anti-APPL antibody [EPR13569] - BSA and Azide free ab250150



#### 6 Images

#### Overview

**Product name** Anti-APPL antibody [EPR13569] - BSA and Azide free

**Description** Rabbit monoclonal [EPR13569] to APPL - BSA and Azide free

**Host species** Rabbit

**Tested applications** Suitable for: IHC-P, ICC/IF, WB

Reacts with: Human **Species reactivity** 

Predicted to work with: Mouse, Rat

**Immunogen** Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: HeLa, MCF7 and HEK-293 cell lysates. IHC-P: Human pancreas and colon carcinoma

tissue. ICC/IF: MCF7 cells.

ab250150 is the carrier-free version of ab180140. **General notes** 

> Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.

This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cellbased assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar<sup>®</sup> is a trademark of Fluidigm Canada Inc.

This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free production

For more information see here.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit

#### **Properties**

Form Liquid

**Storage instructions** Shipped at 4°C. Store at +4°C. Do Not Freeze.

Storage buffer pH: 7.2

Constituent: PBS

Carrier free Yes

Purity Affinity purified
Clonality Monoclonal
Clone number EPR13569

**Isotype** IgG

#### **Applications**

The Abpromise guarantee Our Abpromise guarantee covers the use of ab250150 in the following tested applications.

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

Application	Abreviews	Notes
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 90 kDa (predicted molecular weight: 80 kDa).

#### **Target**

**Function** Required for the regulation of cell proliferation in response to extracellular signals from an early

endosomal compartment. Links Rab5 to nuclear signal transduction.

**Tissue specificity** High levels in heart, ovary, pancreas and skeletal muscle.

Sequence similarities Contains 1 PH domain.

Contains 1 PID domain.

**Domain** Overexpression of an N-terminal domain (residues 1-319) or a C-terminal region (residues 273-

709) has a proapoptotic effect.

Post-translational

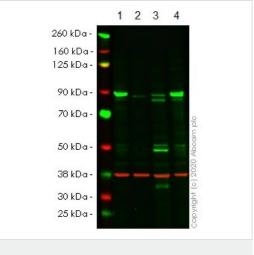
modifications

Phosphorylated upon DNA damage, probably by ATM or ATR.

**Cellular localization** Early endosome membrane. Nucleus. Early endosomal membrane-bound and nuclear.

Translocated into the nucleus upon release from endosomal membranes following internalization

of EGF.



Western blot - Anti-APPL antibody [EPR13569] - BSA and Azide free (ab250150)

All lanes: Anti-APPL antibody [EPR13569] (ab180140) at 1/1000 dilution

Lane 1: Wild-type HeLa cell lysate

Lane 2: APPL CRISPR/Cas9 edited HeLa cell lysate

Lane 3: MCF7 cell lysate

Lane 4: HEK-293T cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

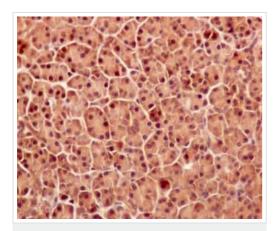
Predicted band size: 80 kDa Observed band size: 90 kDa

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab180140).

Lanes 1-4: Merged signal (red and green). Green - <u>ab180140</u> observed at 90 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (ab8245) observed at 37 kDa.

ab180140 was shown to react with Anti-APPL in wild-type HeLa cells in western blot. The band observed in CRISPR/Cas9 edited cell line ab265187 (CRISPR/Cas9 edited cell lysate ab257836) lane below 90kDa may represent truncated forms and cleaved fragments. This has not been investigated further. Wild-type HeLa and APPL1 CRISPR/Cas9 edited HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk.

ab180140 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated overnight at 4°C at a 1 in 10000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-APPL antibody

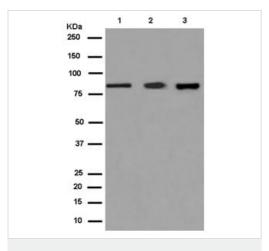
[EPR13569] - BSA and Azide free (ab250150)

This data was developed using <u>ab180140</u>, the same antibody clone in a different buffer formulation.Immunohistochemical analysis of paraffin-embedded Human pancreas carcinoma tissue labeling APPL with <u>ab180140</u> at 1/100 dilution. Secondary antibody Goat anti rabbit lgG(HRP) prediluted. Counter stain Hematoxylin. Perform heat mediated antigen retrieval with EDTA buffer pH 9 before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-APPL antibody [EPR13569] - BSA and Azide free (ab250150)

This data was developed using <u>ab180140</u>, the same antibody clone in a different buffer formulation.lmmunofluorescent analysis of 4% paraformaldehyde fixed MCF7 cells labeling APPL with <u>ab180140</u> at 1/500 dilution. Secondary antibody Goat anti rabbit lgG(Dylight 555) Conterstained with Dapi blue.



Western blot - Anti-APPL antibody [EPR13569] - BSA and Azide free (ab250150)

**All lanes :** Anti-APPL antibody [EPR13569] (**ab180140**) at 1/10000 dilution

Lane 1 : HeLa cell lysate

Lane 2 : MCF7 cell lysate

Lane 3 : 293 cell lysate

Lysates/proteins at 20 µg per lane.

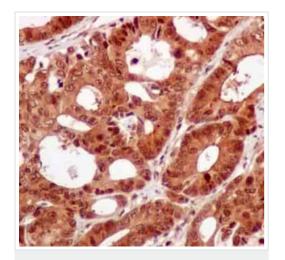
#### Secondary

**All lanes :** Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated at 1/1000 dilution

**Predicted band size:** 80 kDa **Observed band size:** 90 kDa

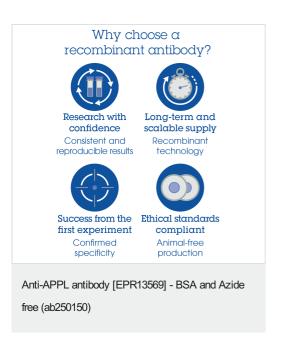
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Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-APPL antibody

[EPR13569] - BSA and Azide free (ab250150)



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

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