abcam

ab318136

Anti-POLE (mutated P286R) antibody [EPR28640-56]

Recombinant | RabMAb | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogens	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR28640-56
Purification technique	Affinity purification Protein A
Specificity	We have not tested this antibody on endogenous material. We welcome any feedback from customers who have used this antibody in cell lines or tissue.
Concentration	0.536 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

WB

Tested

Species	Transfected cell lysate - Human
Dilution info	-

Notes	-

Predicted

Species	Human
Dilution info	-
Notes	-

Not recommended

Species	Synthetic peptide - Human
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Transfected cell lysate - Human
Dilution info	-
Notes	-

Predicted

Species	Human
Dilution info	-
Notes	-

Not recommended

Species	Synthetic peptide - Human
Dilution info	-
Notes	-

IHC-P

Tested

Species	Transfected cell lysate - Human
Dilution info	-
Notes	-

Predicted

Species	Human
Dilution info	-
Notes	-

Not recommended

Species	Synthetic peptide - Human
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Transfected cell lysate - Human
Dilution info	-
Notes	-

Predicted

Species	Human
Dilution info	-
Notes	-

Not recommended

Species	Synthetic peptide - Human
Dilution info	-
Notes	-

IP

Tested

Species	Transfected cell lysate - Human
Dilution info	-
Notes	-

Predicted

Species	Human
Dilution info	-
Notes	-

Not recommended

Species	Synthetic peptide - Human
Dilution info	-
Notes	-

Dot

Tested

Species	Synthetic peptide - Human
Dilution info	-
Notes	-

Predicted

Species	Human
Dilution info	-
Notes	-

Not recommended

Species	Transfected cell lysate - Human

Dilution info	-
Notes	-

Target data

See full target information POLE P286R

Function	Catalytic component of the DNA polymerase epsilon complex (PubMed:10801849). Participates in chromosomal DNA replication (By similarity). Required during synthesis of the leading DNA strands at the replication fork, binds at/or near replication origins and moves along DNA with the replication fork (By similarity). Has 3'-5' proofreading exonuclease activity that corrects errors arising during DNA replication (By similarity). Involved in DNA synthesis during DNA repair (PubMed:20227374, PubMed:27573199). Along with DNA polymerase POLD1 and DNA polymerase POLK, has a role in excision repair (NER) synthesis following UV irradiation (PubMed:20227374).

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

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

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	The POLE protein also known as DNA polymerase epsilon catalytic subunit A functions as an important component in DNA replication and repair mechanisms. POLE possesses a molecular mass of approximately 261 kDa and demonstrates expression primarily in proliferating cells such as those in the bone marrow lymphoid tissues and the gastrointestinal tract. It operates within the eukaryotic DNA polymerase family distinct from other polymerases due to its high fidelity in DNA synthesis which minimizes errors during replication.
Biological function summary	The POLE protein plays an essential role in the high-fidelity synthesis of leading-strand DNA during replication. POLE is part of a larger complex known as the DNA polymerase epsilon holoenzyme collaborating with accessory proteins that enhance its ability to synthesize DNA accurately. Moreover this protein contributes significantly to the proofreading function as it has intrinsic 3' to 5' exonuclease activity allowing the removal of incorrectly paired nucleotides therefore preventing mutations.
Pathways	The POLE protein is fundamentally involved in the DNA replication and repair pathways. These pathways ensure genomic integrity and stability important for normal cell division and preventing genomic instability associated with cancer. POLE interacts with other proteins such as PCNA and RFC (replication factor C) within these pathways facilitating efficient replication fork progression and repair processes. POLE's activity ensures coordination with leading strand synthesis complementing the functions of other polymerases involved in lagging strand DNA synthesis.
Associated diseases and disorders	Mutations in the POLE gene have been linked to colorectal cancer and endometrial cancer. These conditions are often characterized by a hypermutated phenotype arising from impaired DNA proofreading ability due to ineffective exonuclease activity. In this context POLE mutations can assume significance in sporadic cancer cases as well as in hereditary cancer syndromes where it interacts with mismatch repair proteins highlighting its role in maintaining genomic fidelity.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

We do not recommend this combination. It is not covered by our product promise.

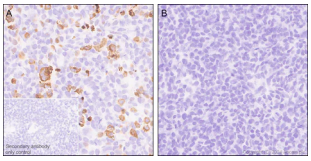
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
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6 product images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-POLE (mutated P286R) antibody [EPR28640-56] (ab318136)

Immunohistochemical analysis of paraffin-embedded (A) 293T cells transfected with a human POLE (mutated P286R) expression vector containing a myc-His-tag®. (B) 293T cells transfected with a human wild-type POLE expression vector containing a myc-His-tag®. tissue labeling POLE (mutated P286R) with ab318136 at 1/2000 (0.268 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

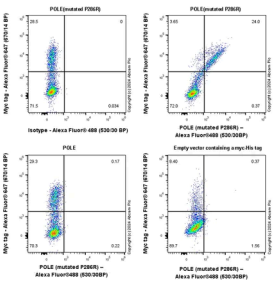
Positive staining on (A) 293T cells transfected with a human POLE (mutated P286R) expression vector containing a myc-His-tag®, no staining on (B) 293T cells transfected with a human wild-type POLE expression vector containing a myc-His-tag®.
The section was incubated with ab318136 for 30 mins at room temperature.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument
Incubate slides with 3% Hydrogen Peroxide for 10 mins at room temperature after secondary antibody incubation to reduce the background

Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins

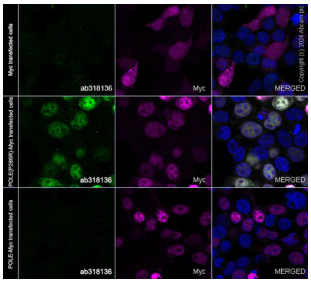
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Flow Cytometry (Intracellular) - Anti-POLE (mutated P286R) antibody [EPR28640-56] (ab318136)

Flow cytometric analysis of 4% paraformaldehyde fixed 0.1% Tween-20 permeabilized 293T (human embryonic kidney epithelial cell) transfected with a human POLE (mutated P286R) containing a myc-His-tag® (upper right) / 293T transfected with a human wild-type POLE expression vector containing a myc-His-tag® (bottom left) / 293T transfected with an empty vector containing a myc-His-tag® (bottom right). cells labelling POLE (mutated P286R) with ab318136 at 1/500 dilution (0.1ug)/ Upper right, bottom left and bottom right (Red) compared with a Rabbit monoclonal IgG (ab172730) / Left isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue).

Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/5000 dilution was used as the secondary antibody.



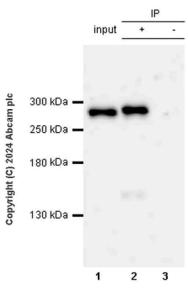
Immunocytochemistry/ Immunofluorescence - Anti-POLE (mutated P286R) antibody [EPR28640-56] (ab318136)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized 293T (human embryonic kidney epithelial cell) cells labelling POLE (mutated P286R) with ab318136 at 1/100 (5.36 ug/ml) dilution, followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 2 ug/mL dilution (Green).

Confocal image showing nuclear staining in 293T cells (shown in green) transfected with a human POLE (mutated P286R) expression vector containing a Myc tag, and negative staining in 293T cells transfected with a wildtype POLE expression vector. The counterstain was observed in magenta. Nuclear DNA was labelled with DAPI (shown in blue). Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

ab223894 Anti-Myc tag mouse monoclonal antibody (Alexa Fluor® 594) was used to counterstain tubulin at 1/100 5ug/ml dilution (Magenta). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 2 ug/mL dilution.



Immunoprecipitation - Anti-POLE (mutated P286R) antibody [EPR28640-56] (ab318136)

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POLE (mutated P286R) was immunoprecipitated from 0.35 mg 293T cells transfected with a human POLE (mutated P286R) expression vector containing a myc-His-tag®, whole cell lysate with ab318136 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab318136 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP) (ab131366) was used at 1/5000 dilution.

Lane 1: 293T cells transfected with a human POLE (mutated P286R) expression vector containing a myc-His-tag®, whole cell lysate
Lane 2: ab318136 IP in 293T cells transfected with a human POLE (mutated P286R) expression vector containing a myc-His-tag®, whole cell lysate
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab318136 in 293T cells transfected with a human POLE (mutated P286R) expression vector containing a myc-His-tag®, whole cell lysate

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Lane 2:

Immunoprecipitation - Anti-POLE (mutated P286R) antibody [EPR28640-56] (ab318136) at 1/30 dilution

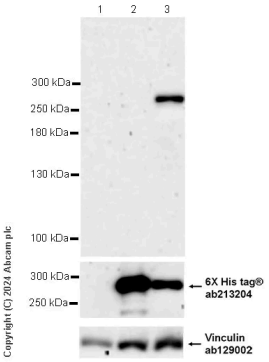
Lane 2:
Immunoprecipitation - Anti-POLE (mutated P286R) antibody [EPR28640-56] - BSA and Azide free (ab318137) at 1/30 dilution

All lanes:
293T cells transfected with a human POLE (mutated P286R) expression vector containing a myc-His-tag®, whole cell lysate

Secondary

All lanes:
Immunoprecipitation - VeriBlot for IP Detection Reagent (HRP) (ab131366) at 1/5000 dilution

Exposure time: 67s



Western blot - Anti-POLE (mutated P286R) antibody [EPR28640-56] (ab318136)

POLE (mutated P286R) Western blot staining using rabbit Anti-POLE (mutated P286R) antibody

In Western blot, Anti-Vinculin antibody [EPR8185] (ab129002) staining at 1/10000 dilution.

In Western blot, Anti-6X His tag® antibody [EPR20547] - ChIP Grade (ab213204) staining at 1/5000 dilution.

All lanes:
Western blot - Anti-POLE (mutated P286R) antibody [EPR28640-56] (ab318136) at 1/1000 dilution

Lane 1:
293T cells transfected with an empty vector containing a myc-His-tag®, whole cell lysate at 20 µg

Lane 2:
293T cells transfected with a human wild-type POLE expression vector containi a myc-His-tag®, whole cell lysate at 20 µg

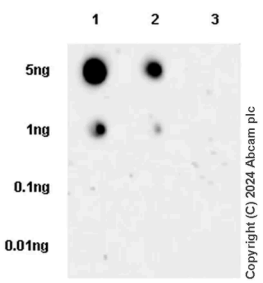
Lane 3:
293T cells transfected with a human POLE (mutated P286R) expression vector containi a myc-His-tag®, whole cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Observed band size: 261 kDa, 124 kDa

Exposure time: 180s



Dot Blot - Anti-POLE (mutated P286R) antibody [EPR28640-56] (ab318136)

POLE (mutated P286R) Dot Blot staining using rabbit Anti-POLE (mutated P286R) antibody

Dot blot analysis of POLE (mutated P286R) using ab318136 at 1:1000 (0.536 ug/ml) followed by a Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (ab97051) at 1:100,000 dilution.

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

Lanes 1 - 3:
Dot Blot - Anti-POLE (mutated P286R) antibody [EPR28640-56] (ab318136) at 1/1000 dilution

Lanes 1 - 3:
Dot Blot - Anti-POLE (mutated P286R) antibody [EPR28640-56] - BSA and Azide free (ab318137) at 1/1000 dilution

Secondary

All lanes:
Dot Blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Exposure time: 180s

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.