abcam

ab300625

Anti-Phospho - (Ser/Thr) Phe antibody [EPR26858-4]

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
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR26858-4
Purification technique	Affinity purification Protein A
Concentration	0.486 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

ELISA

Tested

Species	Modified Amino Acid
Dilution info	0.125 µg/mL
Notes	-

WB

Tested

Species	Modified Amino Acid
Dilution info	1/1000
Notes	-

Dot

Tested

Species	Modified Amino Acid
Dilution info	1/1000
Notes	-

IHC-P

Tested

Species	Modified Amino Acid
Dilution info	1/4000
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

ICC/IF

Tested

Species	Modified Amino Acid
Dilution info	1/4000
Notes	-

Flow Cyt (Intra)

Tested

Species	Modified Amino Acid
Dilution info	1/50

Notes	-

IP

Tested

Species	Modified Amino Acid
Dilution info	1/30
Notes	-

Target data

See full target information Phospho - (Ser/Thr) Phe

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	Phospho-(Ser/Thr) Phe commonly referenced as phosphorylated serine or threonine and sometimes noted as phospho plays an important role mechanically in signal transduction processes by modifying protein function through phosphorylation. It is not a singular protein but rather refers to a specific post-translational modification wherein a phosphate group is added to serine or threonine residues of proteins. Given its nature it does not have a fixed molecular mass. This phosphorylation occurs in various cell types including muscle brain and liver cells as the proteins involved are ubiquitous components of signaling pathways across different tissues.
Biological function summary	The addition of a phosphate group to serine or threonine residues alters protein conformation affecting enzyme activity protein-protein interactions and cellular localization. This modification participates in protein complexes enabling the proteins to act as switches in anabolic and catabolic processes. In many cellular tasks phospho-antibodies detect these modified residues allowing researchers to delineate protein activity states in cellular signaling.
Pathways	Phospho-(Ser/Thr) Phe holds significant importance in the MAPK and AKT signaling pathways. Proteins such as RAF MEK and ERK in the MAPK pathway and AKT and mTOR in the AKT pathway are heavily regulated by serine/threonine phosphorylation. This regulation ensures proper cellular responses to stimuli like growth factors and stress linking extracellular signals to cellular responses.
Associated diseases and disorders	Dysregulation of phospho-(Ser/Thr) Phe modifications can lead to conditions like cancer and neurodegenerative diseases. In cancer for example aberrant phosphorylation in the MAPK pathway involving proteins such as BRAF and KRAS contributes to uncontrolled cell proliferation. Similarly in neurodegenerative diseases improper phosphorylation of proteins like tau can result in toxicity leading to a cascade of cellular dysfunction and disease progression.

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.

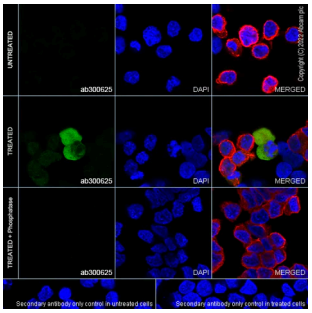
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

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:
Terms & Conditions.

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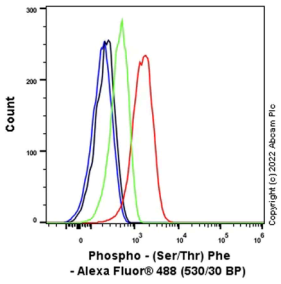
13 product images



Immunocytochemistry/ Immunofluorescence - Anti-Phospho - (Ser/Thr) Phe antibody [EPR26858-4] (ab300625)

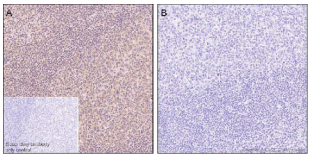
Phospho - (Ser/Thr) Phe Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Phospho - (Ser/Thr) Phe antibody

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized HEK-293 (human embryonic kidney epithelial cell) cells labelling Phospho - (Ser/Thr) Phe with ab300625 at 1/4000 (0.118 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 increased cytoplasmic and nuclear staining in HEK-293 cell line treated with Calyculin A (50 nM) for 3 h, then the signal decreased after phosphatase treatment at 37°C for 2h. is observed. ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 2.5 ug/ml dilution (Red). 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.



Flow Cytometry (Intracellular) - Anti-Phospho - (Ser/Thr) Phe antibody [EPR26858-4] (ab300625)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized HEK-293 (human embryonic kidney epithelial cell) treated with 50nM Calyculin A for 3 hours (Red) / Untreated control (Green) cells labelling Phospho - (Ser/Thr) Phe with ab300625 at 1/50 dilution (1ug) (Red) and Green (Red) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/2000 dilution was used as the secondary antibody.

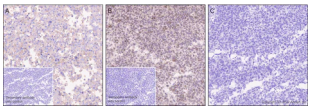


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Phospho - (Ser/Thr) Phe antibody [EPR26858-4] (ab300625)

Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling Phospho - (Ser/Thr) Phe with ab300625 at 1/4000 dilution (0.118 µg/mL), followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Nuclear and cytoplasmic staining on human tonsil without alkaline phosphatase (image A). No signal was detected when tissues were

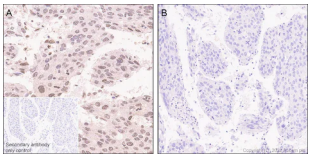
treated with alkaline phosphatase (image B). The section was incubated with ab300625 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.
Secondary antibody only control: Secondary antibody is ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) and used without primary.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins was used.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Phospho - (Ser/Thr) Phe antibody [EPR26858-4] (ab300625)

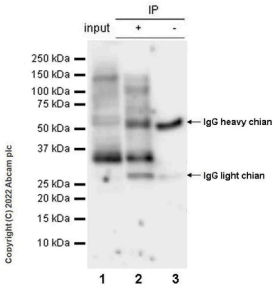
Immunohistochemical analysis of paraffin-embedded (A) HEK-293 (human e tissue labeling Phospho - (Ser/Thr) Phe with ab300625 at 1/4000 (0.118 ug/ml) followed by a ready to use LeicaDS9800 (Bond☒ Polymer Refine Detection) was used. Weak cytoplasmic staining on HEK-293 (human embryonic kidney epithelial cell) cell pellet (without alkaline phosphatase treatment) (image A). Nuclear and cytoplasmic staining on HEK-293 treated with 50nM Calyculin A for 3 hours cell pellet (without alkaline phosphatase treatment) (image B). No signal was detected HEK-293 treated with 50nM Calyculin A for 3 hours cell pellet (alkaline phosphatase treated) (image C). The section was incubated with ab300625 for 30 mins at room temperature.The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.
Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond☒ Polymer Refine Detection).Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Phospho - (Ser/Thr) Phe antibody [EPR26858-4] (ab300625)

Immunohistochemical analysis of paraffin-embedded human hepatocellular tissue labeling Phospho - (Ser/Thr) Phe with ab300625 at 1/4000 dilution (0.118 µg/mL), followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Nuclear and cytoplasmic staining on human hepatocellular carcinoma without alkaline phosphatase (image A). No signal was detected when tissues were treated with alkaline phosphatase (image B). The section was incubated with ab300625 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.
Secondary antibody only control: Secondary antibody is ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) and added without primary.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins was used.



Immunoprecipitation - Anti-Phospho - (Ser/Thr) Phe antibody [EPR26858-4] (ab300625)

Phospho - (Ser/Thr) Phe was immunoprecipitated from 0.35 mg HEK-293 (human embryonic kidney epithelial cell) treated with 50 nM Calyculin A for 3 hours, whole cell lysate with ab300625 at 1/30 dilution (2 µg in 0.35 mg lysates). Western blot was performed on the immunoprecipitate using ab300625 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP)(ab131366) was used at 1/5000 dilution.
Blocking and dilution buffer and concentration: 5% NFDM/TBST

This blot was developed using a high sensitivity ECL substrate. The high-sensitivity ECL substrate used allows for the detection of proteins in the mid-femtogram range.
All lanes:
Immunoprecipitation - Anti-Phospho - (Ser/Thr) Phe antibody [EPR26858-4] (ab300625) at 1/1000 dilution

Lane 1:
HEK-293 (human embryonic kidney epithelial cell) treated with 50nM Calyculin A for 3 hours, whole cell lysate at 10 µg

Lane 2:
ab300625 at 1/30 IP in HEK-293 treated with 50nM Calyculin A for 3 hours, whole cell lysate at 10 µg

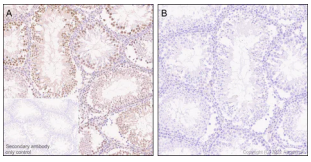
Lane 3:
Rabbit monoclonal IgG (ab172730) instead of ab300625 in HEK-293 treated with 50 nM Calyculin A for 3 hours, whole cell lysate at 10 µg

Secondary

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

Developed using the ECL technique.

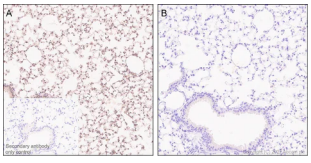
Exposure time: 3min



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Phospho - (Ser/Thr) Phe antibody [EPR26858-4] (ab300625)

Immunohistochemical analysis of paraffin-embedded Rat testis tissue labeling Phospho - (Ser/Thr) Phe with ab300625 at 1/4000 (0.118 ug/ml) followed by a ready to use Rabbit specific IHC polymer detection kit HRP/Dab (ab209101). Nuclear and cytoplasmic staining was observed on rat testis without alkaline phosphatase (image A). No signal was detected when tissues were treated with alkaline phosphatase (image B). The section was incubated with ab300625 for 30 mins at room temperature.The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin. Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/Dab (ab209101).Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins

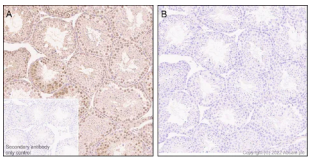
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Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Phospho - (Ser/Thr) Phe antibody [EPR26858-4] (ab300625)

Immunohistochemical analysis of paraffin-embedded mouse lung tissue labeling Phospho - (Ser/Thr) Phe with ab300625 at 1/4000 dilution (0.118 µg/mL), followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Nuclear and cytoplasmic staining was observed on mouse lung without alkaline phosphatase (image A). No signal was detected when tissues were treated with alkaline phosphatase (image B). The section was incubated with ab300625 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.
Secondary antibody only control: Secondary antibody is ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) and used without primary.

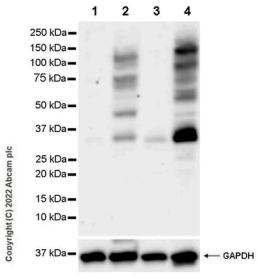
Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins was used.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Phospho - (Ser/Thr) Phe antibody [EPR26858-4] (ab300625)

Immunohistochemical analysis of paraffin-embedded mouse testis tissue labeling Phospho - (Ser/Thr) Phe with ab300625 at 1/4000 dilution (0.118 µg/mL), followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Nuclear and cytoplasmic staining was observed on mouse testis without alkaline phosphatase (image A). No signal was detected when tissues were treated with alkaline phosphatase (image B). The section was incubated with ab300625 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.
Secondary antibody only control: Secondary antibody is ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) and used without primary.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins was used.



Western blot - Anti-Phospho - (Ser/Thr) Phe antibody [EPR26858-4] (ab300625)

Phospho - (Ser/Thr) Phe Western blot staining using rabbit Anti-Phospho - (Ser/Thr) Phe antibody

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

Observed MW(kDa): Multiple bands.

All lanes:
Western blot - Anti-Phospho - (Ser/Thr) Phe antibody [EPR26858-4] (ab300625) at 1/1000 dilution

Lane 1:
Untreated HeLa (human cervix adenocarcinoma epithelial cell), whole cell lysate at 20 µg

Lane 2:
HeLa treated with 100nM Calyculin A for 30 minutes, whole cell lysate at 20 µg

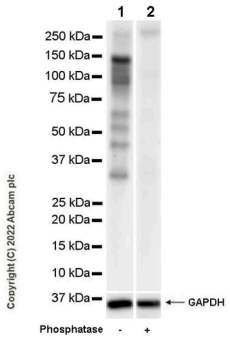
Lane 3:
Untreated HEK-293 (human embryonic kidney epithelial cell), whole cell lysate at 20 µg

Lane 4:
HEK-293 treated with 50nM Calyculin A for 3 hours, whole cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051)

Exposure time: 103s



Western blot - Anti-Phospho - (Ser/Thr) Phe antibody [EPR26858-4] (ab300625)

Blocking and dilution buffer and concentration: 5% NFDM/TBST
Observed MW(kDa): Multiple bands.

This blot was developed using a high sensitivity ECL substrate. The high-sensitivity ECL substrate used allows for the detection of proteins in the mid-femtogram range.

All lanes:
Western blot - Anti-Phospho - (Ser/Thr) Phe antibody [EPR26858-4] (ab300625) at 1/1000 dilution

Lane 1:
HEK-293 (human embryonic kidney epithelial cell) treated with 50nM Calyculin A for 3 hours, whole cell lysate at 20 µg

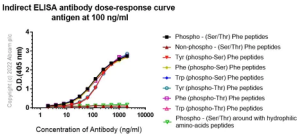
Lane 2:
HEK-293 treated with 50nM Calyculin A for 3 hours, whole cell lysate (phosphatase treated membrane) at 20 µg

Secondary

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

Developed using the ECL technique.

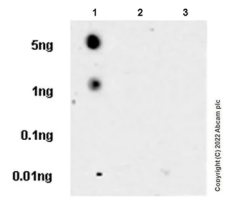
Exposure time: 48s



ELISA - Anti-Phospho - (Ser/Thr) Phe antibody [EPR26858-4] (ab300625)

Indirect ELISA antibody dose-response curve using ab300625 between 0-1000 ng/mL. Antigen concentration of 100 ng/mL. An alkaline phosphatase-conjugated AffiniPure goat anti-rabbit IgG (H+L) (1/2500) was used as the secondary antibody.

Phospho-(Ser/Thr) Phe peptides: a mixture of (Tyr/Phe/Trp)-Phospho - (Ser/Thr) Phe peptides.



Dot Blot - Anti-Phospho - (Ser/Thr) Phe antibody [EPR26858-4] (ab300625)

Dot blot analysis of Phospho - (Ser/Thr) Phe using ab300625 at 1:1000 (0.473 µg/ml) followed by a Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (ab97051) at 1:10,000 dilution. Lane 1: Phospho - (Ser/Thr) Phe peptides Lane 2: Non-phospho - (Ser/Thr) Phe peptides Lane 3: Phospho - (Ser/Thr) (Lys/Arg/Asp/Glu) peptides Exposure time: 3 minutes Blocking and diluting buffer and concentration: 5% NFDM/TBST

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