abcam

Product datasheet

Anti-METTL1 (phospho S27) antibody [EPR24280-9] ab271062

Recombinant RabMAb

10 Images

Overview

Product name	Anti-METTL1 (phospho S27) antibody [EPR24280-9]
Description	Rabbit monoclonal [EPR24280-9] to METTL1 (phospho S27)
Host species	Rabbit
Tested applications	**Suitable for:** WB, Flow Cyt (Intra), ICC/IF, IP, Dot blot **Unsuitable for:** IHC-P
Species reactivity	**Reacts with:** Mouse, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HEK-293T, HeLa, RAW264.7 cell lysates ICC/IF: RAW 264.7, HeLa cells Flow Cyt: HeLa cells IP: HEK-293T cells
General notes	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 monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

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 long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal

Clone number	EPR24280-9
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab271062 in the following tested applications.

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

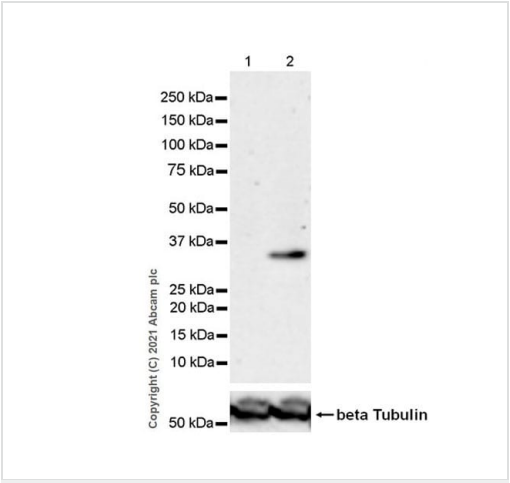
Application	Abreviews	Notes
WB		1/1000. Predicted molecular weight: 31 kDa.
Flow Cyt (Intra)		1/500.
ICC/IF		1/50.
IP		1/30.
Dot blot		1/1000.

Application notes Is unsuitable for IHC-P.

Target

Function	Catalyzes the formation of N(7)-methylguanine at position 46 (m7G46) in tRNA.
Tissue specificity	Ubiquitous.
Pathway	tRNA modification; N(7)-methylguanine-tRNA biosynthesis.
Sequence similarities	Belongs to the methyltransferase superfamily. TrmB family.
Post-translational modifications	Phosphorylation at Ser-27 inactivates its catalytic activity but does not affect the interaction with WDR4.
Cellular localization	Nucleus.

Images



Western blot - Anti-METTL1 (phospho S27) antibody [EPR24280-9] (ab271062)

All lanes : Anti-METTL1 (phospho S27) antibody [EPR24280-9] (ab271062) at 1/1000 dilution

Lane 1 : Untreated RAW264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate
Lane 2 : RAW264.7 treated with 100 nM Calycin A for 30 minutes, whole cell lysate

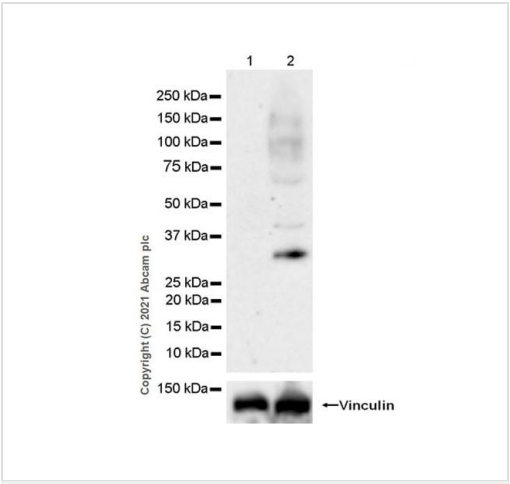
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) at 1/20000 dilution

Predicted band size: 31 kDa
Observed band size: 31 kDa

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

Exposure time: 3 minutes



Western blot - Anti-METTL1 (phospho S27) antibody [EPR24280-9] (ab271062)

All lanes : Anti-METTL1 (phospho S27) antibody [EPR24280-9] (ab271062) at 1/1000 dilution

Lane 1 : Untreated HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate
Lane 2 : HeLa treated with 100 nM Calycin A for 30 minutes, whole cell lysate

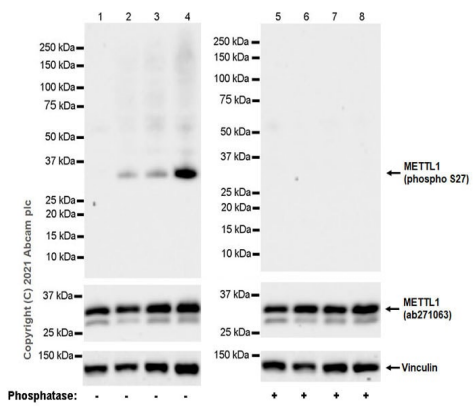
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) at 1/20000 dilution

Predicted band size: 31 kDa
Observed band size: 31 kDa

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

Exposure time: 3 minutes



Western blot - Anti-METTL1 (phospho S27) antibody [EPR24280-9] (ab271062)

All lanes : Anti-METTL1 (phospho S27) antibody [EPR24280-9] (ab271062) at 1/1000 dilution

Lane 1 : HEK-293T (human embryonic kidney epithelial cell) whole cell lysate (Untreated membrane)
Lane 2 : HEK-293T treated with 100 nM Calycin A for 30 minutes, whole cell lysate (Untreated membrane)
Lane 3 : HEK-293T treated with /ml PDGF-AA for 5 minutes then treated with 100 nM Calyculin A for 30 minutes, whole cell lysate (Untreated membrane)
Lane 4 : HEK-293T transfected with an AKT1 expression vector, and treated with 100 ng/ml PDGF-AA for 5 minutes then treated with 100 nM Calyculin A for 30 minutes, whole cell lysate (Untreated membrane)
Lane 5 : HEK-293T whole cell lysate (phosphatase treated membrane)
Lane 6 : HEK-293T treated with 100 nM Calycin A for 30 minutes, whole cell lysate (phosphatase treated membrane)
Lane 7 : HEK-293T treated with /ml PDGF-AA for 5 minutes then treated with 100 nM Calyculin A for 30 minutes, whole cell lysate (phosphatase treated membrane)
Lane 8 : HEK-293T transfected with an AKT1 expression vector, and treated with /ml PDGF-AA for 5 minutes then treated with 100 nM Calyculin A for 30 minutes, whole cell lysate (phosphatase treated membrane)

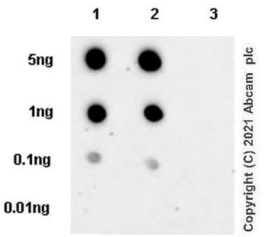
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 31 kDa
Observed band size: 31 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBSTAKT1 in lane 4 and lane 8 act as a kinase to active METTL1 (phospho S27).

Exposure time: 26 seconds

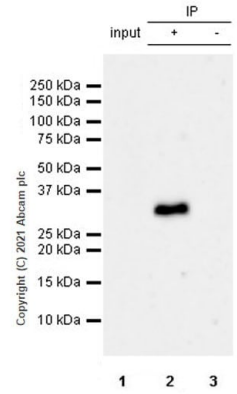


Dot Blot - Anti-METTL1 (phospho S27) antibody [EPR24280-9] (ab271062)

Dot blot analysis of METTL1 (phospho S27) using ab271062 at 1:1000 (0.547 ug/ml) followed by a Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) at 1:100,000 dilution.

Exposure time: 3 minutes

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



Immunoprecipitation - Anti-METTL1 (phospho S27) antibody [EPR24280-9] (ab271062)

METTL1 (phospho S27) was immunoprecipitated from 0.35 mg RAW264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) treated with 100 nM Calyculin A for 30 minutes, whole cell lysate with ab271062 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab271062 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP)(**ab131366**) was used at 1/5000 dilution.

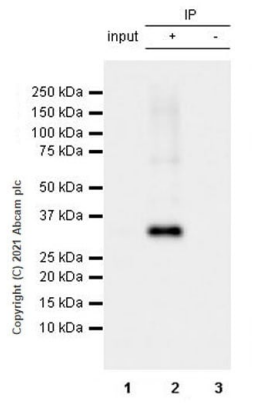
Lane 1: RAW264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) treated with 100 nM Calyculin A for 30 minutes, whole cell lysate 10 ug

Lane 2: ab271062 IP in RAW264.7 treated with 100 nM Calyculin A for 30 minutes, whole cell lysate

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab271062 in RAW264.7 treated with 100 nM Calyculin A for 30 minutes, whole cell lysate

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

Exposure time: 50 seconds



Immunoprecipitation - Anti-METTL1 (phospho S27) antibody [EPR24280-9] (ab271062)

METTL1 (phospho S27) was immunoprecipitated from 0.35 mg HEK-293T (human embryonic kidney epithelial cell) transfected with an AKT1 expression vector, and treated with 100ng/ml PDGF-AA for 5 minutes then treated with 100 nM Calyculin A for 30 minutes, whole cell lysate with ab271062 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab271062 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP)(**ab131366**) was used at 1/5000 dilution.

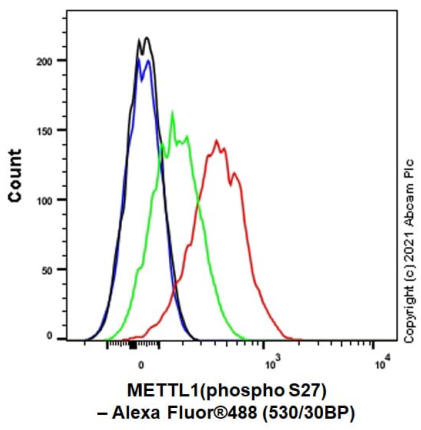
Lane 1: HEK-293T (human embryonic kidney epithelial cell) transfected with an AKT1 expression vector, and treated with 100ng/ml PDGF-AA for 5 minutes then treated with 100 nM Calyculin A for 30 minutes, whole cell lysate 10 ug

Lane 2: ab271062 IP in HEK-293T transfected with an AKT1 expression vector, and treated with 100ng/ml PDGF-AA for 5 minutes then treated with 100 nM Calyculin A for 30 minutes, whole cell lysate

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab271062 in HEK-293T transfected with an AKT1 expression vector, and treated with 100ng/ml PDGF-AA for 5 minutes then treated with 100 nM Calyculin A for 30 minutes, whole cell lysate

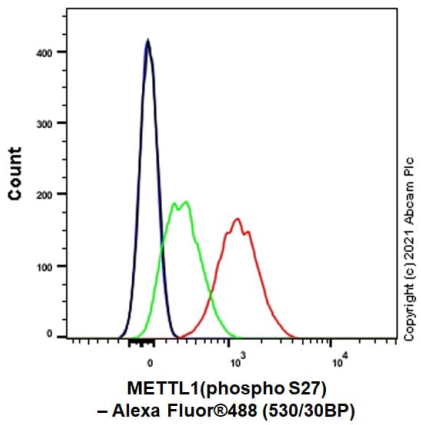
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 3.25 seconds



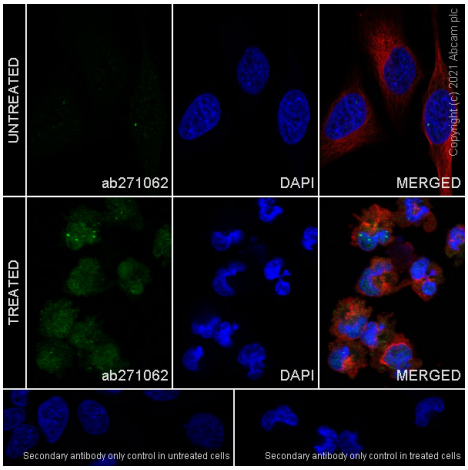
Flow Cytometry (Intracellular) - Anti-METTL1 (phospho S27) antibody [EPR24280-9] (ab271062)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized RAW 264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) treated with 100nM Calyculin A for 5min (Red) / Untreated control (Green) cells labelling METTL1 (phospho S27) with ab271062 at 1/500 dilution (0.1ug)/ Red and Green 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.



Flow Cytometry (Intracellular) - Anti-METTL1 (phospho S27) antibody [EPR24280-9] (ab271062)

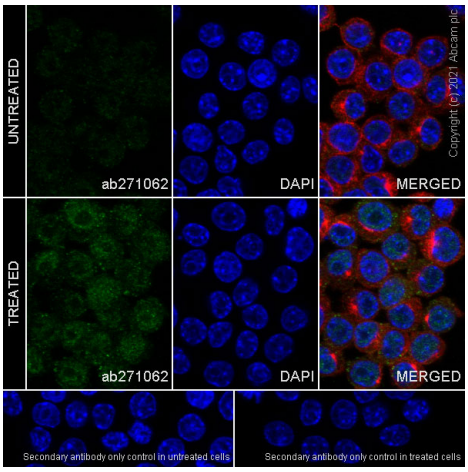
Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized HeLa (human cervix adenocarcinoma epithelial cell) treated with 100nM Calyculin A for 5min (Red) / Untreated control (Green) cells labelling METTL1 (phospho S27) with with ab271062 at 1/500 dilution (0.1ug)/ Red and Green 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.



Immunocytochemistry/ Immunofluorescence - Anti-METTL1 (phospho S27) antibody [EPR24280-9] (ab271062)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized HeLa cells labelling METTL1 (phospho S27) with ab271062 at 1/50 dilution, followed by **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 dilution (Green). Confocal image showing increased cytoplasmic and nuclear staining in HeLa cells treated with Calyculin A (100 nM) for 5 min. is observed. **ab195889** Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 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 dilution.



Immunocytochemistry/ Immunofluorescence - Anti-METTL1 (phospho S27) antibody [EPR24280-9] (ab271062)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized RAW 264.7 cells labelling METTL1 (phospho S27) with ab271062 at 1/50 dilution, followed by **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 dilution (Green). Confocal image showing increased cytoplasmic and nuclear staining in RAW 264.7 cells treated with Calyculin A (100 nM) for 5 min. is observed. **ab195889** Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 dilution (Red). The Nuclear counterstain was DAPI (Blue).

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