abcam

ab315887

Anti-Puromycin antibody [EPR27218-173]

Anti-Puromycin antibody [EPR27218-173] (ab315887) is a rabbit monoclonal antibody detecting Puromycin in **Western Blot, Flow Cytometry (Intra), IP, IHC-P, ICC/IF**.

- Biophysical QC for unrivalled batch-batch consistency

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	EPR27218-173
Purification technique	Affinity purification Protein A
Concentration	0.525 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Chemical
Dilution info	1/5000

Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

ICC/IF

Tested

Species	Chemical
Dilution info	1/500
Notes	-

WB

Tested

Species	Chemical
Dilution info	1/1000
Notes	-

Flow Cyt (Intra)

Tested

Species	Chemical
Dilution info	1/500
Notes	-

IP

Tested

Species	Chemical
Dilution info	1/30
Notes	-

Target data

See full target information Puromycin

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

What is this antibody validated in?
Anti-Puromycin antibody [EPR27218-173] (ab315887) is a rabbit recombinant monoclonal antibody and is validated for use in Western Blot (WB), Flow Cytometry (Intra), Immunoprecipitation (IP), Immunohistochemistry (IHC-P), Immunocytochemistry/immunofluorescence (ICC/IF).

Other related products
We have a range of other formats of antibody clone [EPR27218-173] also available for your convenience: ab315887, Carrier free - ab315888, Alexa Fluor® 647 - ab322729, Alexa Fluor® 488 - ab323262

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.

Trial sizes available!
Test your antibody or perform pre-screening before committing to a larger quantity. Sold in 20µl. Discover our selection of trial-size antibodies.

Supplementary info

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

Activity summary	Puromycin is a well-known antibiotic that acts by disrupting protein synthesis. It mimics the structure of an aminoacyl-tRNA leading to premature termination of the growing polypeptide chain during translation. Puromycin is small with a molecular mass of approximately 471 daltons. It interacts mainly within the cytoplasm where it can incorporate into the peptide chain due to its similarity to the 3' end of the aminoacyl-tRNA. Puromycin does not have a specific expression site like endogenous proteins because it is not naturally occurring in animals; rather it is used experimentally.
Biological function summary	The function of puromycin is to interfere with protein synthesis by targeting ribosomes. It binds to the ribosomal A site leading to the release of incomplete polypeptides. Puromycin is not part of a complex but establishes a strong interaction with ribosomes found in prokaryotic and eukaryotic cells. This interaction gives it unique utility in experiments that require inhibition of translation such as studies involving protein turnover and synthesis rates.
Pathways	The action of puromycin strongly impacts protein synthesis pathways. It inhibits the elongation stage of the translation pathway by mimicking an aminoacyl-tRNA. Puromycin does not involve complex pathways itself but its effects can influence other proteins involved in translation such as elongation factors and the ribosomal subunits. This makes puromycin useful in basic scientific research to study how cells control protein synthesis.
Associated diseases and disorders	Puromycin's ability to inhibit translation links it to studies on cancer and neurodegenerative diseases. Researchers use puromycin to explore how disrupting protein synthesis can affect cancer cell growth. It serves as a model for understanding toxins that affect similar pathways in neurodegenerative disorders. Through these studies connections have been made between puromycin's action and proteins involved in these diseases such as oncogenes and neuromodulatory proteins which are key to understanding disease progression and developing potential treatments.

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

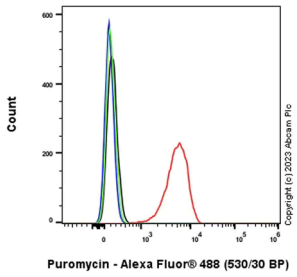
We do not recommend this combination. It is not covered by our product promise.

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.

8 product images

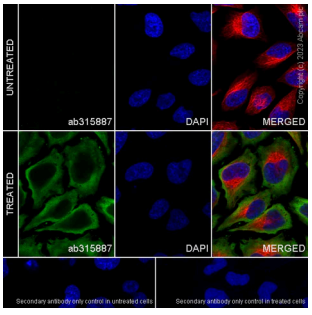


Flow Cytometry (Intracellular) - Anti-Puromycin antibody [EPR27218-173] (ab315887)

Puromycin Flow Cytometry (Intracellular) staining of HeLa (human cervical adenocarcinoma epithelial cell) treated with 1uM Puromycin for 30min (Red) / Untreated HeLa (Green) using rabbit Anti-Puromycin antibody

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized HeLa (human cervical adenocarcinoma epithelial cell) treated with 1uM Puromycin for 30min (Red) / Untreated HeLa (Green) cells labelling Puromycin with ab315887 at 1/500 dilution (0.1 ug)/Red (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).

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



Immunocytochemistry/ Immunofluorescence - Anti-Puromycin antibody [EPR27218-173] (ab315887)

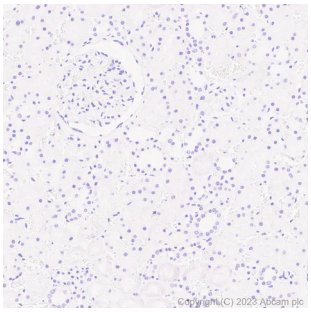
Puromycin Immunocytochemistry/ Immunofluorescence staining of HeLa (human cervical adenocarcinoma epithelial cell) using rabbit Anti-Puromycin antibody

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized HeLa (human cervical adenocarcinoma epithelial cell) cells labelling Puromycin with ab315887 at 1/500 (1.048 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 cytoplasmic staining in HeLa cells after treatment with 1 uM Puromycin for 30 mins, while showing no staining in untreated HeLa cells.
Image was taken with a confocal microscope(Leica-Microsystems, TCS SP8).

ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 2.5ug/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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Puromycin antibody [EPR27218-173] (ab315887)

Puromycin Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human kidney using rabbit Anti-Puromycin antibody

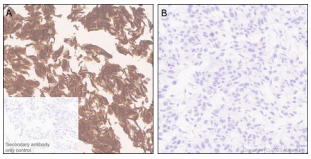
Immunohistochemical analysis of paraffin-embedded Human kidney tissue labeling Puromycin with ab315887 at 1/100 (5.24 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Negative control: no staining on human kidney. The section was incubated with ab315887 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 was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Puromycin antibody [EPR27218-173] (ab315887)

Puromycin Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Puromycin antibody

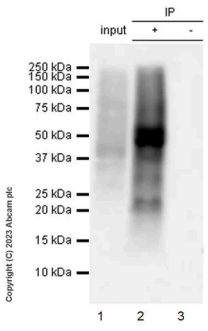
Immunohistochemical analysis of paraffin-embedded Panel A HeLa (human cervical adenocarcinoma epithelial cell) cell pellet treated with Puromycin (1 uM) for 30min; Panel B untreated HeLa cell pellet tissue labeling Puromycin with ab315887 at 1/5000 (0.105 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Positive staining on (A) HeLa cell pellet treated with Puromycin (1 uM) for 30min; no staining on (B) untreated HeLa cell pellet. The section was incubated with ab315887 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 was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins



Immunoprecipitation - Anti-Puromycin antibody [EPR27218-173] (ab315887)

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

Puromycin was immunoprecipitated from 0.35 mg HeLa (human cervical adenocarcinoma epithelial cell) treated with 1uM Puromycin for 30min whole cell lysate with ab315887 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab315887 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP)(ab131366) was used at 1/5000 dilution.

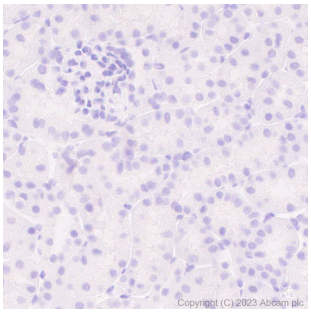
Lane 1: HeLa (human cervical adenocarcinoma epithelial cell) treated with 1uM Puromycin for 30min whole cell lysate
Lane 2: ab315887 IP in HeLa (human cervical adenocarcinoma epithelial cell) treated with 1uM Puromycin for 30min whole cell lysate
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab315887 in HeLa treated with 1uM Puromycin for 30min whole cell lysate
All lanes:
Immunoprecipitation - Anti-Puromycin antibody [EPR27218-173] (ab315887) at 1/30 dilution

All lanes:
HeLa (human cervical adenocarcinoma epithelial cell) treated with 1uM Puromycin for 30min whole cell lysate

Secondary

All lanes:
Immunoprecipitation - VeriBlot for IP Detection Reagent (HRP) (ab131366) at 1/1000 dilution

Exposure time: 8s



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Puromycin antibody [EPR27218-173] (ab315887)

Puromycin Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Mouse kidney using rabbit Anti-Puromycin antibody

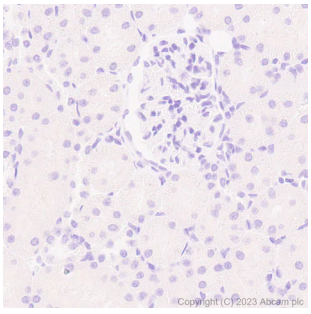
Immunohistochemical analysis of paraffin-embedded Mouse kidney tissue labeling Puromycin with ab315887 at 1/100 (5.24 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Negative control: no staining on mouse kidney. The section was incubated with ab315887 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 was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Puromycin antibody [EPR27218-173] (ab315887)

Puromycin Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Rat kidney using rabbit Anti-Puromycin antibody

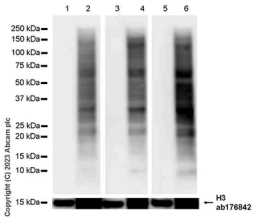
Immunohistochemical analysis of paraffin-embedded Rat kidney tissue labeling Puromycin with ab315887 at 1/100 (5.24 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Negative control: no staining on rat kidney. The section was incubated with ab315887 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 was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins



Western blot - Anti-Puromycin antibody [EPR27218-173] (ab315887)

Puromycin Western blot staining using rabbit Anti-Puromycin antibody

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

Puromycin is a naturally occurring aminonucleoside antibiotic that inhibits protein synthesis by ribosome-catalyzed incorporation into the C-terminus of elongating nascent chains, blocking further extension and resulting in premature termination of translation.

In Western blot, Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842) staining at 1/100000 dilution.

Exposure time: Lane 1-2: 1 second, Lane 3-4: 6 seconds, Lane 5-6: 3 seconds.

All lanes:
Western blot - Anti-Puromycin antibody [EPR27218-173] (ab315887) at 1/1000 dilution

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

Lane 2:
HeLa treated with 1uM Puromycin for 30min whole cell lysate at 20 µg

Lane 3:
Untreated NIH/3T3 (mouse embryonic fibroblast) whole cell lysate at 20 µg

Lane 4:
NIH/3T3 treated with 1uM Puromycin for 30min whole cell lysate at 20 µg

Lane 5:
Untreated C6 (rat glial tumor glial cell) whole cell lysate at 20 µg

Lane 6:
C6 treated with 1uM Puromycin for 30min whole cell lysate at 20 µg

Secondary

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

Observed band size: 10-250 kDa, 15 kDa

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