DATASHEET

Puromycin Recombinant Rabbit Monoclonal Antibody(ARA482)

CAT. NO. ARA5792

KEY FEATURES

Target	Puromycin	**Source / Host**	Rabbit
Reactivity	Species independent	**Clonality**	Monoclonal
Applications	WB,IHC,IF/ICC,FC	**Conjugation**	Unconjugated
Isotype	IgG	**Storage**	at -20°C

BACKGROUND

Puromycin is an aminonucleoside antibiotic, derived from the Streptomyces alboniger bacterium, that causes premature chain termination during translation taking place in the ribosome. Part of the molecule resembles the 3' end of the aminoacylated tRNA. It enters the A site and transfers to the growing chain, causing the formation of a puromycylated nascent chain and premature chain release. The exact mechanism of action is unknown at this time but the 3' position contains an amide linkage instead of the normal ester linkage of tRNA. That makes the molecule much more resistant to hydrolysis and stops the ribosome.

APPLICATION

To ensure optimal assay performance, AREX recommends conducting reagent titration tailored to each testing system for optimal detection results.

WB	1:1,000 - 1:2,500
IHC	1:10,000 - 1:20,000
IF/ICC	1:2,000 - 1:5,000
FC	1 μg/mL

*Results are sample-specific. Please refer to your local assay conditions and test parameters for reference.

OVERVIEW

Description	Recombinant rabbit monoclonal antibody to Puromycin for WB, IHC, IF/ICC and FC.
Specificity	Recognizes puromycin incorporated into nascent polypeptide chains.
Antibody Type	Primary antibody,Recombinant
Molecular Weight	Observed: 14-250 kDa
Purification	Protein A affinity purification
Isotype	IgG
Form/Buffer	Liquid in PBS (pH 7.4) with 0.1% BSA, 40% glycerol and 0.2% ProClean 950.

*AREX continuously optimizes our products. Webpage content may not reflect the latest updates. For inquiries, please contact info@arexbio.com or your local distributor.

*Clone Number, Reactivity, Source/Host and Clonality can be found in the product name and Key Features section above.

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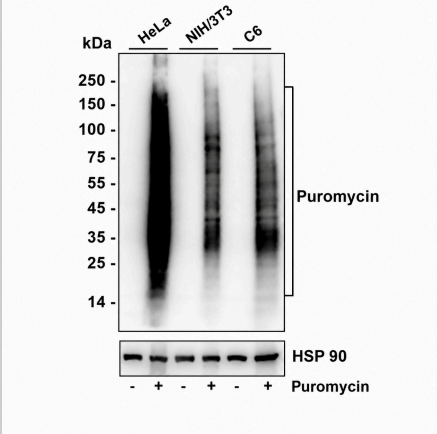
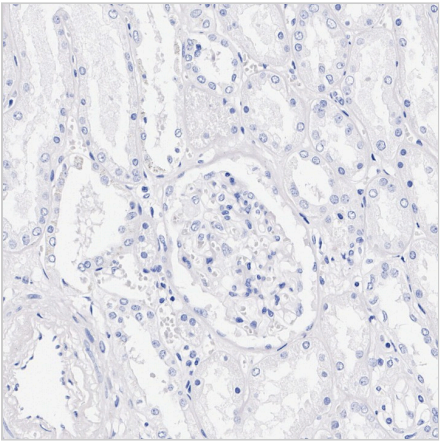


Western blot analysis of Puromycin on different lysates with Puromycin Recombinant Rabbit Monoclonal Antibody(ARA482) at 1:2,500 dilution.

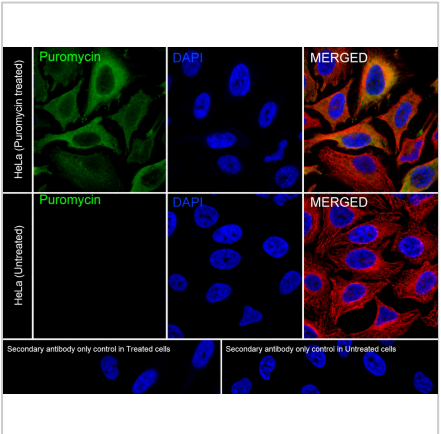
Lane 1: Untreated HeLa cell lysate
Lane 2: HeLa treated with 1 µM Puromycin for 30 minutes cell lysate
Lane 3: Untreated NIH/3T3 cell lysate
Lane 4: NIH/3T3 treated with 1 µM Puromycin for 30 minutes cell lysate
Lane 5: Untreated C6 cell lysate
Lane 6: C6 treated with 1 µM Puromycin for 30 minutes cell lysate

Lysates/proteins at 10 µg/lane.
Exposure time: 46 seconds. Blocking: 5% NFDM/TBST for 1 hour at room temperature. The primary antibody at 1:2,500 dilution was used overnight at 4°C. HRP-conjugated Goat Anti-Rabbit IgG (H+L) at 1:50,000 dilution was used for 1 hour at room temperature.
Observed band size: 14-250 kDa



Immunohistochemical analysis of paraffin-embedded human kidney tissue (negative control) with Puromycin Recombinant Rabbit Monoclonal Antibody(ARA482) at 1:20,000 dilution.

Antigen retrieval was performed by heat mediation with Tris-EDTA buffer (pH 9.0) for 20 minutes at 95°C. Endogenous peroxidase was blocked with 3% H2O2 for 10 minutes at room temperature. The section was blocked with 1% BSA + 10% normal goat serum for 10 minutes at room temperature and then probed with the primary antibody for 1 hour at room temperature.



Immunofluorescent analysis of 4% PFA-fixed HeLa cells treated with 1 µM Puromycin for 30 minutes with Puromycin Recombinant Rabbit Monoclonal Antibody(ARA482) at 1:5,000 dilution.

Cells were permeabilized with 0.1% Triton X-100 for 15 minutes and blocked with 1% BSA + 10% normal goat serum for 1 hour at room temperature. The primary antibody was incubated overnight at 4°C, followed by AREX® Fluor 488-conjugated Goat Anti-Rabbit IgG (H+L) for 45 minutes at room temperature. β-tubulin was stained in red and nuclei were counterstained with DAPI (blue).

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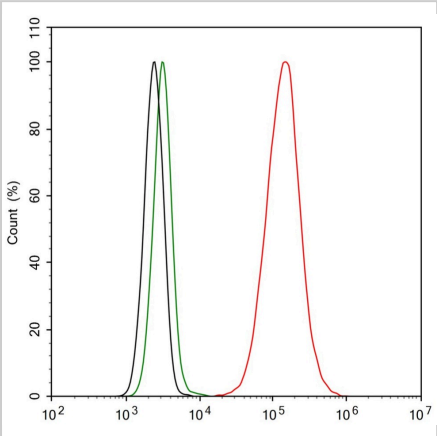

Flow cytometry (intracellular) analysis of 4% PFA-fixed HeLa cells treated with 1 µM Puromycin for 30 minutes with Puromycin Recombinant Rabbit Monoclonal Antibody(ARA482) at 1 µg/mL (red), compared with Rabbit IgG isotype control (green).

Cells were permeabilized with 90% methanol for 5 minutes and blocked with 1% BSA + 10% normal goat serum for 15 minutes at room temperature. The primary antibody was incubated for 15 minutes at room temperature, followed by AREX® Fluor 488-conjugated Goat Anti-Rabbit IgG (H+L) for 15 minutes at room temperature.

STORAGE

Store at 4°C short term. For long term storage, store at -20°C, avoiding freeze/thaw cycles.

NOTE

For Research Use Only. Not for diagnostic, therapeutics, prophylactic or in vivo use.

More information: www.arexbio.com