abcam

Product datasheet

Anti-1-methyladenosine (m1A) antibody [EPR-19836-208] ab208196

Recombinant RabMAb

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Overview

Product name	Anti-1-methyladenosine (m1A) antibody [EPR-19836-208]
Description	Rabbit monoclonal [EPR-19836-208] to 1-methyladenosine (m1A)
Host species	Rabbit
Specificity	Has been developed to discriminate between the modified base 1-methyladenosine (m1A) and the unmodified counterpart Adenosine (A).
Tested applications	**Suitable for:** IP, Dot blot, ELISA
Species reactivity	**Reacts with:** Species independent
Immunogen	Chemical/ Small Molecule. This information is proprietary to Abcam and/or its suppliers.
Positive control	IP: 5' Biotin-mN-mN-mN-mN-mN-[m1A]-mN-mN-mN-mN-mN 3'. ELISA: BSA-conjugated m1A-modified nucleotide.
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: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified

| Clonality	Monoclonal
Clone number	EPR-19836-208
Isotype	IgG

Applications

The Abpromise guarantee   Our **Abpromise guarantee** covers the use of ab208196 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
IP		Use at an assay dependent concentration. Use 0.2 µg.
Dot blot		Use a concentration of 2 µg/ml.
ELISA		Use a concentration of 0.005 - 4 µg/ml.

Target

Relevance   N1-methyladenosine (m1A) is a RNA modification that has been reported in mRNA, tRNA, rRNA and lncRNA. It is found in bacteria, archaea and eukaryotes. The addition of the methyl group to the nitrogen at the 1st position of the adenosine base gives it a positive charge.

Images

Dot Blot - Anti-1-methyladenosine (m1A) antibody [EPR-19836-208] (ab208196)
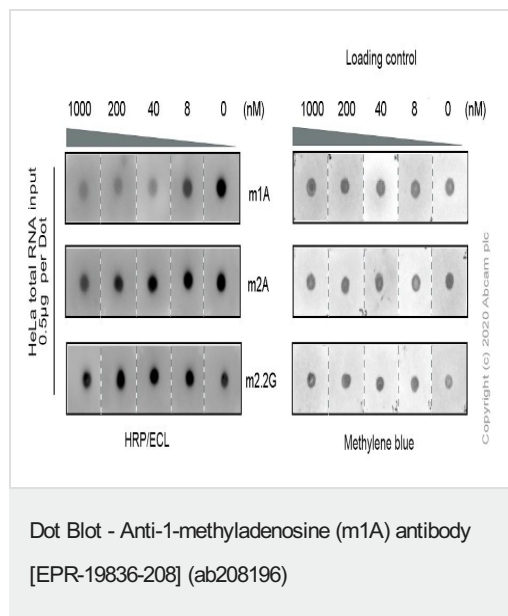
Primary antibody dilution: 1/500

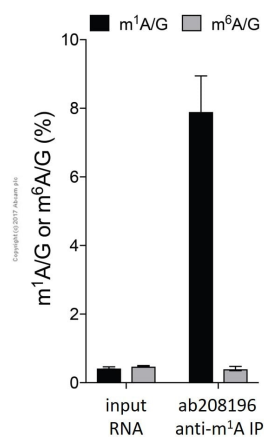Secondary antibody: Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated

Secondary antibody dilution: 1/20,000

Blocking buffer and dilution buffer: AdvanBlockTM Chemi Blocking buffer

Input: HeLa total RNA 0.5 µg per Dot

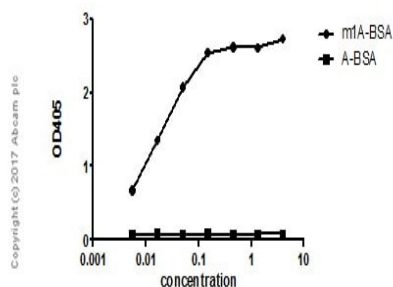Competitive nucleosides: m1A, m2A, m2.2G

Exposure time: 37 seconds

Immunoprecipitation - Anti-1-methyladenosine (m1A) antibody [EPR-19836-208] (ab208196)
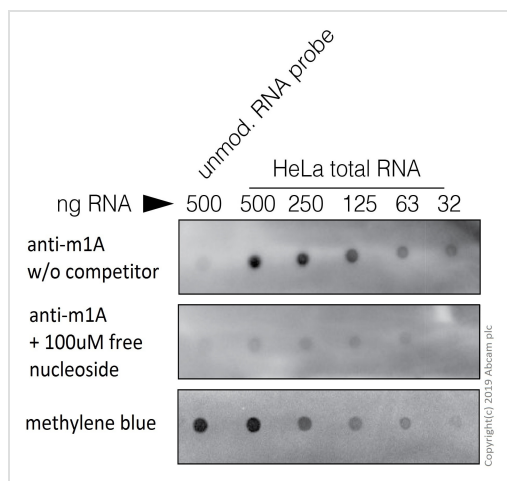
This image is courtesy of Dr Sigrid Nachtergaele, University of Chicago.

m1A was immunoprecipitated from 20 µg of HeLa (Human cervix adenocarcinoma epithelial cell) polyA+ RNA with 10 µg of ab208196 and 40 µL of Protein G dynabeads per sample (the IP buffer was 50mM Tris-HCl pH 7.4, 150mM NaCl, and 0.1% NP-40). The amount of m1A was quantified relative to the level of G by LC-MS/MS with electrospray ionization and in positive ionization mode, and compared to the level of m6A/G in the same samples. Error bars represent technical replicate injections of the same sample in mass spec.

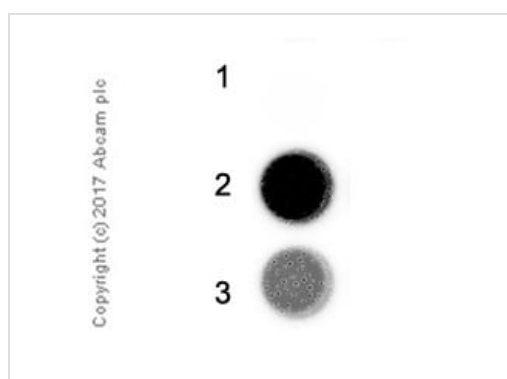ELISA - Anti-1-methyladenosine (m1A) antibody [EPR-19836-208] (ab208196)

BSA-conjugated m1A (modified) and A (unmodified) nucleosides were coated onto wells of a 96 well plate. ELISA was performed on 1.0 µg/ml of antigen using ab208196 at a concentration range of 0.005-4.000 µg/ml, followed by Goat Anti-Rabbit IgG, (H+L), alkaline phosphatase conjugated secondary antibody at 1/2500 dilution.

Dot Blot - Anti-1-methyladenosine (m1A) antibody
[EPR-19836-208] (ab208196)

This image is courtesy of Dr Sigrid Nachtergaele,
University of Chicago.

Dot blot of total RNA using ab208196 at 2 ug/mL. The Amersham Hybond N+ membrane was pre-spotted with 500, 250, 125, 63 and 32 ng/dot of HeLa total RNA and 500ng of an unmodified RNA probe. The membrane was then blocked with 5% BSA in TBS with 0.1% Tween-20. Followed by blotting with anti-m1A ab208196, or ab208196 together with 100uM of free m1A nucleoside in the same blocking solution, to inhibit m1A binding. A goat anti-rabbit HRP was used as the secondary antibody at 1:5000 dilution. Methylene blue stain was used to verify RNA loading.

Immunoprecipitation - Anti-1-methyladenosine (m1A) antibody [EPR-19836-208] (ab208196)

The IP was performed in a U-bottom non-adsorbing propylene 96-well plate.

ab208196 (0.2 µg) was coated into Dynabeads® sheep-anti-rabbit IgG (50 µl) for 1h at RT.

Unmodified/modified oligonucleotides (5 µM) were added to samples containing the antibody/bead complexes and incubated with agitation for 1 hour at RT.

After washing, Peroxidase-conjugated Streptavidin was incubated at 1/1000 dilution with agitation for 1 hour at RT.

ECL substrate was then added and the results read in a non-transparent 96-well plate with a digital detector and analyzed using ImageJ.

Lane 1: Buffer only.

Lane 2: Modified oligonucleotide (5 µM), 5' Biotin-mN.mN.mN.mN.mN.[m1A].mN.mN.mN.mN.mN 3'

Lane 3: Unmodified oligonucleotide (5 µM), 5' Biotin-mN.mN.mN.mN.mN.[A]*.mN.mN.mN.mN.mN 3'

N - equimolar mixture of (A/U/G/C)
m - 2'O methyl protection
* - phosphorothioate protection

Blocking buffer and concentration: 5% NFDM/TBST

Dilution buffer and concentration: TBST/0.1% Triton X-100/1 mM EDTA

Why choose a recombinant antibody?

Research with confidence
Consistent and reproducible results

Long-term and scalable supply
Recombinant technology

Success from the first experiment
Confirmed specificity

Ethical standards compliant
Animal-free production

Anti-1-methyladenosine (m1A) antibody [EPR-19836-208] (ab208196)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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