abcam

Product datasheet

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

リコンビナント RabMAb

4 References 画像数 6

製品の概要

製品名	Anti-1-methyladenosine (m1A) antibody [EPR-19836-208]
製品の詳細	Rabbit monoclonal [EPR-19836-208] to 1-methyladenosine (m1A)
由来種	Rabbit
特異性	Has been developed to discriminate between the modified base 1-methyladenosine (m1A) and the unmodified counterpart Adenosine (A).
アプリケーション	**適用あり:** IP, Dot blot, ELISA
種交差性	**交差種:** Species independent
免疫原	Chemical/ Small Molecule. This information is proprietary to Abcam and/or its suppliers.
ポジティブ・コントロール	IP: 5' Biotin-mN-mN-mN-mN-mN-[m1A]-mN-mN-mN-mN-mN 3'. ELISA: BSA-conjugated m1A-modified nucleotide.
特記事項	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**.

製品の特性

製品の状態	Liquid
保存方法	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.
バッファー	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
精製度	Protein A purified

ポリ/モノ	モノクローナル
クローン名	EPR-19836-208
アイソタイプ	IgG

アプリケーション

The Abpromise guarantee **Abpromise保証は、** 次のテスト済みアプリケーションにおけるab208196の使用に適用されます

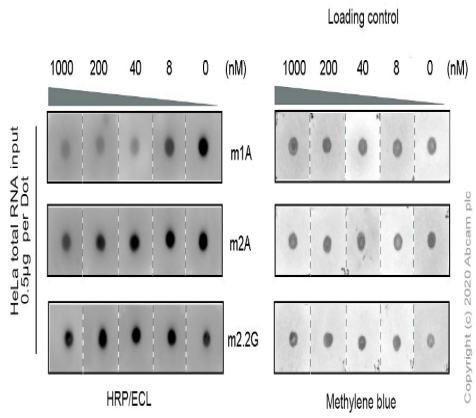
アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
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.

ターゲット情報

関連性 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.

画像



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

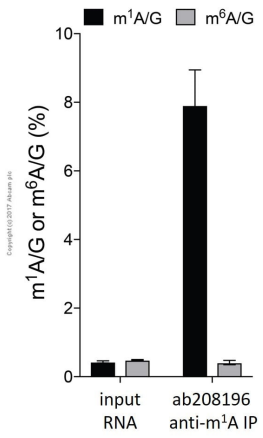
Secondary antibody dilution: 1/20,000

Blocking buffer and dilution buffer: AdvanBlockTM Chemi Blocking buffer

Input: HeLa total RNA 0.5 µg per Dot

Competitive nucleosides: m1A, m2A, m2.2G

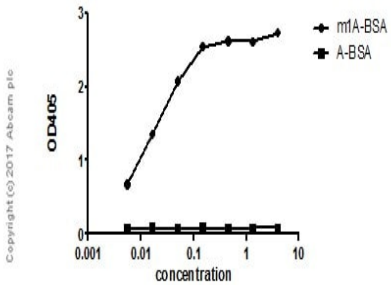
Exposure time: 37 seconds



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

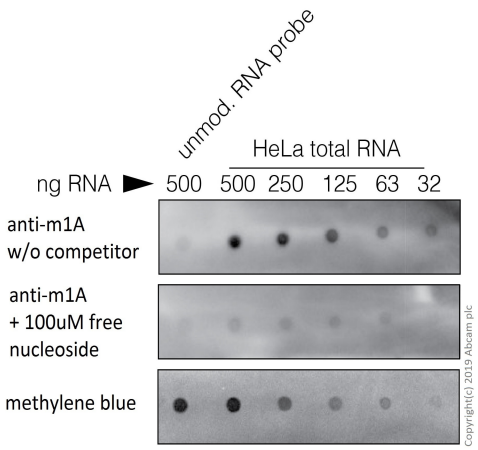
This image is courtesy of Dr Sigrid Nachtergaele, University of Chicago.

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)

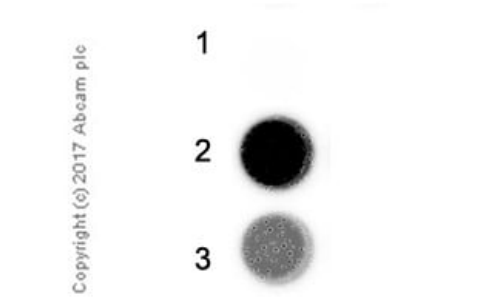
BSA-conjugated m1A (modified) and A (unmodified) nucleosides were coated onto wells of a 96 well plate. ELISA was performed on 1.0 µg/ml of antigen using ab208196 at a concentration range of 0.005-4.000 µg/ml, followed by Goat Anti-Rabbit IgG, (H+L), alkaline phosphatase conjugated secondary antibody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

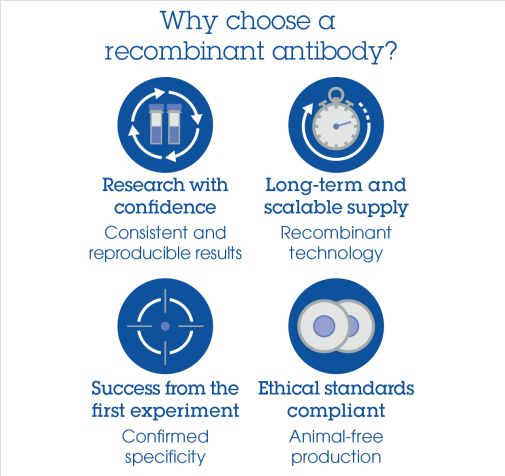
Lane 2: Modified oligonucleotide (5 µM), 5' Biotin-mN.mN.mN.mN.mN.[m1A].mN.mN.mN.mN.mN 3'

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



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