abcam

ab10805

Anti-5-methylcytosine (5-mC) antibody [33D3]

Anti-5-methylcytosine (5-mC) antibody [33D3] (ab10805) is a mouse monoclonal antibody detecting 5-methylcytosine (5-mC) in **Flow Cytometry, IP, IHC-P, IHC-Fr**.

- Over 170 publications
- Trusted since 2005

Key facts

Isotype	IgG1
Host species	Mouse
Storage buffer	pH: 7.4 Constituents: PBS, 0.876% Sodium chloride
Form	Liquid
Clonality	Monoclonal
Immunogen	Chemical / Small Molecule corresponding to 5-Methylcytosine.
Carrier free	Yes
Clone number	33D3
Purification technique	Affinity purification Protein A
Specificity	Modified base 5-methylcytidine found in DNA of plants and vertebrates. Raised against the modified ribonucleoside. Specific for the presence of a methyl group on carbon 5 of the pyrimidine ring.
Light chain type	kappa
Concentration	1 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Expected

Species	Modified Nucleic Acid
Dilution info	Use at an assay dependent concentration.
Notes	-

IHC-P

Tested

Species	Modified Nucleic Acid
Dilution info	-
Notes	Perform heat-mediated antigen retrieval before commencing with IHC staining protocol.

IHC-Fr

Expected

Species	Modified Nucleic Acid
Dilution info	Use at an assay dependent concentration.
Notes	-

ICC

Not recommended

Species	Modified Nucleic Acid
Dilution info	-
Notes	While older lots have performed well in ICC, we have received inconsistent results with the latest lots. Unfortunately, we can no longer guarantee this antibody for use in ICC.

Flow Cyt

Expected

Species	Modified Nucleic Acid
Dilution info	-
Notes	Use 10ul of working dilution to label 1000000 cells in 100ul. (see Habib, M. et al. (1999)). ab170190 - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.

SB

Expected

Species	Modified Nucleic Acid
Dilution info	1/200
Notes	-

Target data

See full target information 5-Methylcytosine

Storage

Shipped at conditions	Blue Ice
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

Anti-5-methylcytosine (5-mC) antibody [33D3] (ab10805) is a mouse monoclonal antibody and is validated for use in Flow Cyt, IHC-Fr, IHC-P, IP, SB in human samples.

Anti-5-methylcytosine (5-mC) antibody [33D3] (ab10805) has been cited over 177 times in peer reviewed journals and is trusted by the scientific community.

Abcam's high quality validation processes ensure Anti-5-methylcytosine (5-mC) antibody [33D3] (ab10805) has high sensitivity and specificity.

Anti-5-methylcytosine (5-mC) antibody [33D3] (ab10805) has 11 independent reviews from customers.

Anti-5-methylcytosine (5-mC) antibody [33D3] (ab10805) specifically detects 5-methylcytosine (5-mC) (UniProt ID: ; Molecular weight: kDa) and is sold in 50 µg selling sizes.

Storage in frost-free freezers is not recommended. Should this product contain a precipitate microcentrifugation before use.While older lots have performed well in ICC, we have received inconsistent results with the latest lots. Unfortunately, we can no longer guarantee this antibody for use in ICC.

Supplementary info

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

Activity summary	5-methylcytosine often referred to as 5-mC 5-methyl cytosine or 5-methylcytidine is a methylated form of the nucleobase cytosine found in the DNA of various organisms. This modification typically occurs at the carbon 5 position of the cytosine ring contributing to the epigenetic regulation of gene expression. While the mass of 5-mC itself is relatively small since it is a modified nucleotide its impact on gene regulation is significant. 5-mC is widely expressed in the genome of higher eukaryotes particularly in CpG dinucleotides where it plays a role in mammalian development and cell differentiation.
Biological function summary	Cytosine methylation affects DNA-protein interactions leading to changes in chromatin structure and the regulation of gene activity. 5-methylcytosine is an important player in the chromatin modification complex where it collaborates with other epigenetic marks to control gene expression profiles. This modified nucleotide helps to stabilize gene silencing maintaining the closed chromatin state which inhibits the transcription machinery's access to specific genomic regions. Such silencing is vital during processes like X-chromosome inactivation and imprinting.
Pathways	Researchers have identified 5-mC within two critical pathways: DNA methylation and demethylation cycle and histone modification pathway. In the DNA methylation pathway 5-mC interacts closely with proteins like DNA methyltransferases (DNMTs) which directly add methyl groups to cytosine bases. In the context of histone modification 5-mC influences the binding of methyl-CpG-binding domain (MBD) proteins which read and interpret methylated DNA marks affecting histone modification and DNA accessibility.
Associated diseases and disorders	The dysregulation of 5-methylcytosine patterns associates prominently with cancer and neurological disorders. Abnormal methylation patterns where 5-mC is either hypermethylated or hypomethylated can lead to the silencing of tumor suppressor genes or activation of oncogenes forming a basis for cancer development. In neurological disorders altered 5-mC levels link to conditions such as Rett syndrome where the dysfunction of the associated protein MeCP2 an important reader of 5-mC contributes to the disease pathology.

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

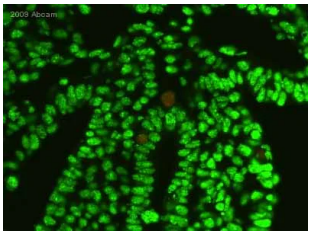
We do not recommend this combination. It is not covered by our product promise.

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.

4 product images



This image is courtesy of a customer review submitted by Ms Sara Maj Wätjen Hyldig

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-5-methylcytosine (5-mC) antibody [33D3] (ab10805)

5-methylcytosine (5-mC) Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using mouse Anti-5-methylcytosine (5-mC) antibody

ab10805 staining 5-Methyl Cytidine in pig embryo (15 to 17 days) tissue section by Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections). Tissue underwent fixation in paraformaldehyde, heat mediated antigen retrieval in Tris-EDTA buffer, permeabilization in Triton X-100 and blocking in 2% BSA for 10 minutes at 25°C. The primary antibody was diluted, 1/100 (PBS + 2% BSA) and incubated with sample for 1 hour at 25°C. An Alexa Fluor® 488 conjugated donkey polyclonal to mouse at 1/250 dilution, was used as secondary.

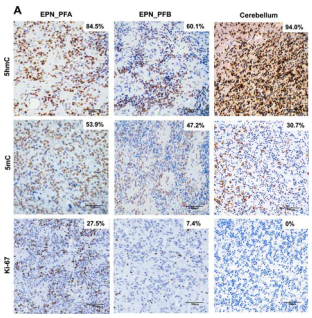


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Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-5-methylcytosine (5-mC) antibody [33D3] (ab10805)

5-methylcytosine (5-mC) Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using mouse Anti-5-methylcytosine (5-mC) antibody

5-methylcytosine immunohistochemistry using Anti-5-methylcytosine (5-mC) antibody [33D3] ab10805. Publication image and figure legend from Wu, T., Zhang, Z. W., et al., 2020, Clin Epigenetics, PubMed: 31992357.

5hmC, 5mC, and Ki-67 index IHC staining in EPN_PF samples and normal cerebellum. a Representative image of 5hmC, 5mC, and Ki-67 staining in EPN_PF and cerebellum. b The Pearson correlation between nuclear positive cells of 5hmC and global 5hmC levels. c The Pearson correlation between Ki-67 index cells and global 5hmC levels. d The Pearson correlation between Ki-67 index and nuclear positive cells of 5hmC. Scale bars represent 100 μm

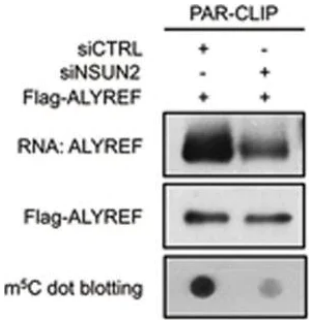


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Dot Blot - Anti-5-methylcytosine (5-mC) antibody [33D3] (ab10805)

5-methylcytosine (5-mC) Dot Blot staining using mouse Anti-5-methylcytosine (5-mC) antibody

5-methylcytosin dot blot using Anti-5-methylcytosine (5-mC) antibody [33D3] ab10805. Publication image and figure legend from Yang, X., Yang, Y., et al., 2017,Cell Res, PubMed 28418038.

NSUN2 regulates nuclear-cytoplasmic shuttling and RNA-binding ability of ALYREF. (A) Immunofluorescence staining of ALYREF (red color) and ASF (green color) upon NSUN2 knockdown (top); line scan graphs (middle) and peak density quantification of line scan graphs (bottom) for ALYREF are also shown. Scale bar, 10 μm. Error bars indicate SEM (n = 120). (B) Western blotting (left) and quantification (right) of nuclear and cytoplasmic distribution of ALYREF in control and NSUN2-knockdown HeLa cells. The protein loading for the cytoplasmic fraction is about 2.5-fold higher than that for the nuclear fraction. PARP1 and TUBULIN serve as nuclear and cytoplasmic markers, respectively. Error bars indicate SEM (n = 3). (C) Immunofluorescence staining of ALYREF (red color) in NSUN2-knockdown HeLa cells transfected with control EGFP (EGFP-EV), EGFP-tagged siNSUN2-insensitive wild-type NSUN2 (EGFP-WT-Ins) or mutant (EGFP-DM-Ins) plasmids (top); line scan graphs (middle) and peak density quantification of line scan graphs (bottom) for ALYREF are also shown. Scale bar, 10 μm. Error bars indicate SEM (n = 120). (D) Western blotting (top) and quantification (bottom) of nuclear and cytoplasmic distribution of ALYREF in NSUN2-knockdown HeLa cells transfected with empty Myc expression vector (Myc-EV), Myc-tagged siNSUN2-insensitive wild-type NSUN2 (Myc-WT-Ins), or Mutant (Myc-DM-Ins). The protein loading for the cytoplasmic fraction is about 2.5-fold higher than that for the nuclear fraction. PARP1 and TUBULIN serve as nuclear and cytoplasmic markers, respectively. Error bars indicate SEM (n = 3). (E) PAR-CLIP assay (left) and quantification (right) of RNA pulled down by Flag-ALYREF upon NSUN2 knockdown. RNA labeled with biotin at 3' end of RNA (End Biotinylation Kit, Thermo) was visualized by the chemiluminescent nucleic acid detection module. m5C-modified RNAs were visualized by dot blotting using m5C antibody. Error bars indicate SEM (n = 3). (F) Rescue PAR-CLIP assay (left) and quantification (right) of RNA pulled down by Flag-ALYREF in NSUN2-knockdown HeLa cells transfected with Myc-EV, Myc-WT-Ins, or Myc-DM-Ins. RNA labeled with biotin at 3' end of RNA (End Biotinylation Kit, Thermo) was visualized by the Chemiluminescent nucleic acid detection module. Error bars indicate SEM (n = 3). P values were calculated by Student's t-test.

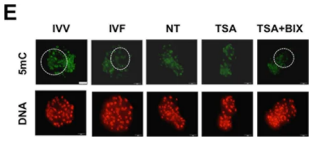


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Immunohistochemistry - Anti-5-methylcytosine (5-mC) antibody [33D3] (ab10805)

5-methylcytosine (5-mC) Immunohistochemistry staining using mouse Anti-5-methylcytosine (5-mC) antibody

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5-methylcytosine immunohistochemistry-immunofluorescence using Anti-5-methylcytosine (5-mC) antibody [33D3] ab10805. Publication image and figure legend from Cao, Z., Hong, R., et al., 2017, PLoS One, Pubmed 28114389.

TSA and BIX-01294 treatment combined restores H3K9me2 and 5mC levels in SCNT embryos.SCNT embryos at the pronuclear stage were cultured in PZM-3 supplemented with 50 nM TSA or 50 nM TSA and 1 μm BIX-01294 for 24 h and SCNT embryos cultured in PZM-3 containing DMSO were served as NT control. Then DMSO, TSA and BIX-01294 in different groups were washed out and embryos were cultured in fresh PZM-3 medium until day7. We isolated and fixed the 4-cell and blastocysts from different groups including NT control, TSA treatment, TSA and BIX-01294 combination treatment for subsequent experiments. At the same time, in vivo fertilized (IVV) and in vitro fertilized (IVF) 4-cell and blastocysts were also collected and fixed. Finally, 4-cell embryos (A) and blastocysts (C) were stained for H3K9me2 (green) and DNA (propidium iodide, red). Bottom panels showed the merged images (yellow) between H3K9me2 signals (green) and DNA staining (red). (E) Blastocysts from all groups were stained for 5mC (green) and DNA (propidium iodide, red). Shown in A, C and E are representative Z-sections obtained by confocal microscopy from one experiment. White dash circle denotes ICM area in C and TE region in E, respectively. Scale bars = 50μm. (B, D) Quantification of the H3K9me2 fluorescence intensity in nucleus of 4-cell and trophectoderm cells. (F) Quantification of the 5mC fluorescence intensity in nucleus of trophectoderm cells. Data in B, D and F are shown as mean ± S.E.M. All experiments above were repeated three times with 20 embryos per group. Values with different superscripts across groups indicate significant differences ($p < < 0.05$) and $*p < < 0.05$.

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