

Datasheet for ABIN1385081

anti-5-Methylcytosine antibody[Go to Product page](#)**1** Validation**2** Images

Overview

Quantity:	100 µL
Target:	5-Methylcytosine
Reactivity:	Please inquire
Host:	Rabbit
Clonality:	Polyclonal
Application:	Western Blotting (WB), Flow Cytometry (FACS), Immunofluorescence (Paraffin-embedded Sections) (IF (p)), Immunohistochemistry (Paraffin-embedded Sections) (IHC (p))

Product Details

Immunogen:	KLH conjugated 5 MethylCytosine
Isotype:	IgG
Cross-Reactivity:	Human
Cross-Reactivity (Details):	5 MethylCytosine
Purification:	Purified by Protein A.

Target Details

Target:	5-Methylcytosine
Alternative Name:	5 MethylCytosine
Target Type:	Chemical
Background:	Synonyms: 5-Methyl Cytidine, 5 m C, 5 mC, 5 me C, 5 Me Cytidine, 5 MeCyd, 5 Methyl Cytidine, 5

Target Details

MethylCytosine, 5-mC, 5-Me Cytidine, 5-Methyl-Cytosine, 5-MethylCytidine.

Background: Cytidine is a nucleoside formed by a cytosine attached to a ribose ring via a beta-N1-glycosidic bond. DNA is methylated on cytidines by DNA methylases (DNMTs) to generate 5-methylcytosine (5-mC), a potent epigenetics marker and regulator of gene expression. The reverse reaction (cytidine demethylation) starts with its oxidation to hydroxymethyl- (5-hmC), formyl- (5-fC), and carboxy- (5-caC) cytidine. Several enzymes, including the Tet family of proteins have been implicated in cytidine demethylation.

Application Details

Application Notes: IHC-P 1:100-500
IF(IHC-P) 1:50-200

Restrictions: For Research Use only

Handling

Format: Liquid

Concentration: 1 µg/µL

Buffer: 0.01M TBS(pH 7.4) with 1 % BSA, 0.02 % Proclin300 and 50 % Glycerol.

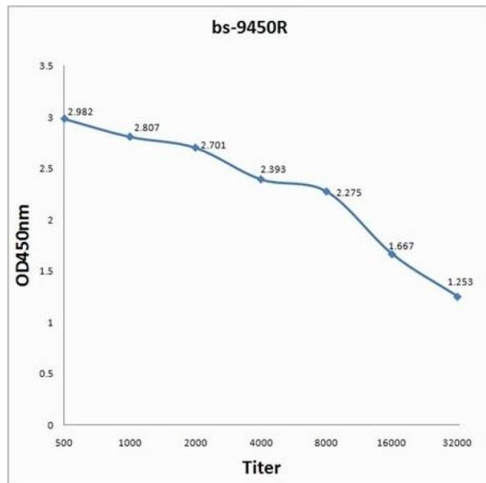
Preservative: Sodium azide

Precaution of Use: This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE, which should be handled by trained staff only.

Storage: 4 °C, -20 °C

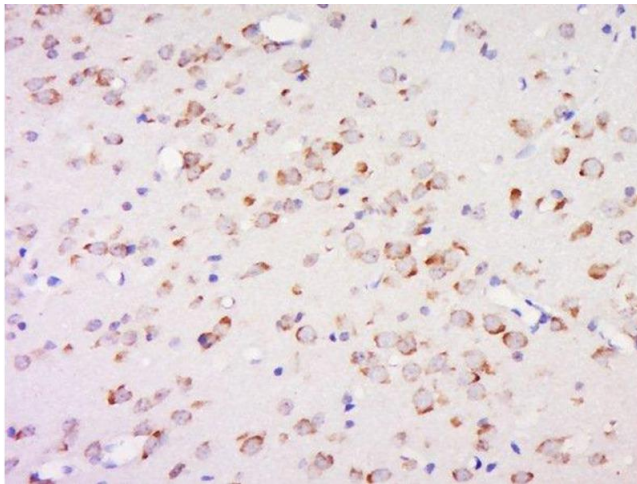
Storage Comment: Shipped at 4°C. Store at -20°C for one year. Avoid repeated freeze/thaw cycles.

Expiry Date: 12 months



ELISA

Image 1. Antigen: 2 µg/100 µL Primary: Antiserum, 1:500, 1:1000, 1:2000, 1:4000, 1:8000, 1:16000, 1:32000; Secondary: HRP conjugated Goat Anti-Rabbit IgG at 1: 5000; TMB staining; Read the data in MicroplateReader by 450nm.



Immunohistochemistry (Paraffin-embedded Sections)

Image 2. Paraformaldehyde-fixed, paraffin embedded (Species/Tissue), Antigen retrieval by boiling in sodium citrate buffer (pH6) for 15min, Block endogenous peroxidase by 3% hydrogen peroxide for 30 minutes, Blocking buffer (normal goat serum) at 37°C for 20min, Antibody incubation with Rabbit Anti-5 MethylCytosine Polyclonal Antibody, Unconjugated at 1:500 overnight at 4°C, followed by a conjugated secondary for 90 minutes and DAB staining.



Successfully validated (Immunohistochemistry (IHC))

by [Prof. Merighi](#), Laboratory of Neurobiology, Department of Veterinary Sciences, University of Turin

Report Number: 104610

Date: Jul 29 2024

Target: 5-Methylcytosine

Lot Number: 9K04V1

Method validated: Immunohistochemistry (IHC)

Positive Control: Adult (> 2 months) CD1 mouse brain fixed in 4% paraformaldehyde.

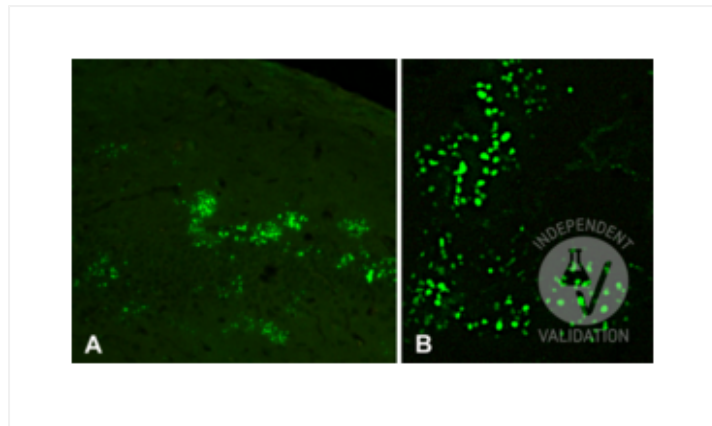
Negative Control: One control slice for each experimental procedure processed omitting the primary antibody; overnight incubation in diluent solution only.

Notes: Passed. The antibody works for IHC-P at 1:100 and 1:200 concentrations without microwave antigen retrieval

Primary Antibody: ABIN1385081

Protocol:

- Perfuse mice with paraformaldehyde 4% in 0.1 M phosphate buffer pH 7.4 and post-fix of samples in the same fixative for an additional 2 h at RT.
- Wash, dehydrate, and embed samples in paraffin wax.
- Wash several times with 0.01 M PBS.
- Cut the brain with a microtome into 6 μ m sections and mount them on glass slides.
- After paraffin removal, sections were incubated for 1 hour at room temperature in PBS containing 1% albumin from chicken egg white (Sigma, A5378) and 0.3% Triton-X-100 (BioRad, 161-0407, lot # 00583) to block non-specific binding sites.
- Incubate sections with the primary antibody anti-5-methyl-cytosine (ABIN1385081; Lot 9K04V1) at 1:50, 1:100, and 1:200 in PBS-BSA-(Sigma, A7906)- PLL (Sigma, P1524) at RT in a humid chamber.
- Wash sections 3x 5 min with 0.01 M PBS.
- Incubate sections with anti-rabbit secondary antibody Alexa Fluor 488, 1:500, in 0.1M PBS (Thermo Fisher Scientific, A11034, lot # 2380031) for 1 h at RT.
- Wash sections 3x 5 min with 0.01 M PBS.
- Specimens were mounted in Fluoroshield (Sigma, F6182, lot # MKCB0153V).
- Images were acquired with a Leica DM 6000B fluorescence microscope with a digital camera at 40x magnification.



Validation image no. 1 for anti-5-Methylcytosine antibody (ABIN1385081)

5-methyl-cytosine immunoreactive neuronal nuclei in the mouse forebrain. A: a group of immunoreactive nuclei in the deep layers of the cerebral cortex (original magnification 20x); B: at higher magnification (40x), the positive 5-methyl-cytosine sites are visible in the form of a punctate reaction. Primary antibody dilution: 1:100. No microwave.