

Datasheet for 200-301-U98**5-mC Antibody****Overview**

Description:	Anti-5-mC (MOUSE) Monoclonal Antibody - 200-301-U98
Item No.:	200-301-U98
Size:	50 µg
Applications:	Dot Blot, IF, FISH
Reactivity:	Human, Mouse, Broad, D. melanogaster
Host Species:	Mouse

Product Details

Background:	5-methyl Cytidine is a modified nucleotide found in the DNA of both plants and vertebrates. Anti-5-mC Antibody is ideal for research in Epigenetics, specifically DNA methylation, due to its applicability to methylation analysis.
Synonyms:	5-methylcytidine, 5-methyl Cytidine
Host Species:	Mouse
Clonality:	Monoclonal
Format:	IgG1

Target Details

Reactivity:	Human, Mouse, Broad, D. melanogaster
Immunogen Type:	Other
Immunogen:	Anti-5-mC Antibody was produced in mice by repeated immunization with 5-methyl Cytidine.
Purity/Specificity:	Anti-5-mC Antibody was purified by Protein A chromatography. Cross-reactivity with 5-mC from other sources has not been determined.

Application Details

Tested Applications:	Dot Blot, IF
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Suggested Applications:	FISH (Based on references)
Application Note:	Anti-5-mC Antibody is tested for IF and Dot Blotting, Fluorescence In Situ Hybridization (FISH), Immunofluorescence and Methylated DNA Immunoprecipitation (MeDIP). Specific conditions for reactivity should be optimized by the end user.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
IF:	1:500
IP:	1-2 µg/IP

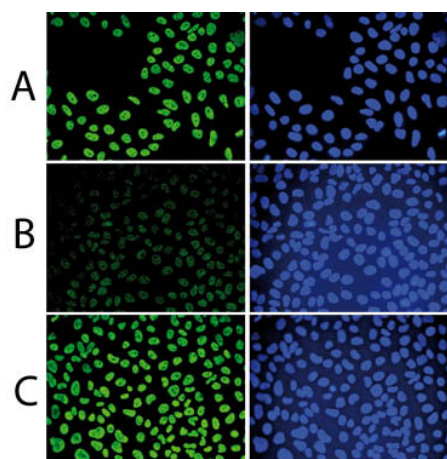
Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	2.4 mg/ml by UV absorbance at 280 nm
Buffer:	0.01 M Sodium Phosphate, 0.25 M Sodium Chloride, pH 7.2
Preservative:	0.05% (w/v) Sodium Azide
Stabilizer:	None

Shipping & Handling

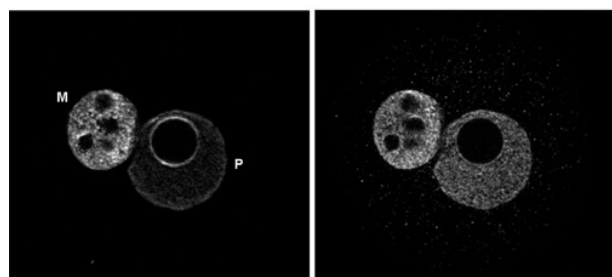
Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
Expiration:	Expiration date is one (1) year from date of receipt.

Images



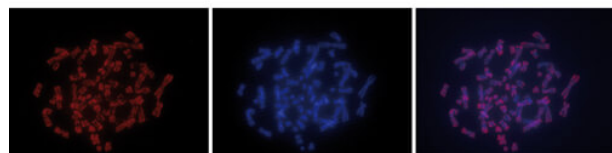
Immunofluorescence Microscopy

Immunofluorescence Microscopy results of Mouse anti-5-mC antibody. Tissue: human osteosarcoma cells (U2OS). Fixation: 2.5% PFA for 30 min. Antigen retrieval: 0.5% Triton X-100 for 1 hour and treated with 2N HCl for 1 hour followed by 2 x 5 minutes with 0.1 M borate buffer to depurinate the DNA. Primary antibody: 5-mC antibody at 1:500. Secondary antibody: Goat anti-Mouse Alexa488 secondary antibody. Staining: (A): 5-mC antibody as green fluorescent signal (left) with DAPI (right). (B): 5-mC antibody with 50 μ M mCTP. (C): 5-mC antibody with 50 μ M hmCTP.



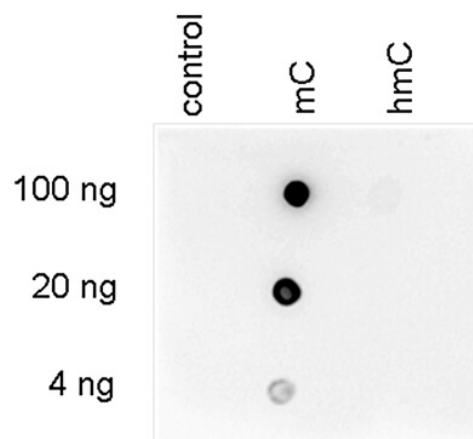
Fluorescence in situ Hybridization (FISH)

Fluorescence in situ Hybridization (FISH) of Mouse Anti-5-mC antibody. Cells: mouse embryo cells. Treatment: none. Fixation: 4% PFA in PBS at 4°C overnight. Block: 0.5% Triton X-100 for 30 min, PBS containing 2% BSA, then 2N HCl. Primary antibody: (Left) anti-5-mC antibody, (Right) anti-single stranded DNA antibody, at 1:200 for 1 hr at RT. Secondary antibody: anti-Mouse CY5 secondary antibody at 1:10,000 for 45 min at RT. Staining: (left) both the maternal (M) and paternal (P) pronuclei are stained with 5-mC antibody. (right) staining of the DNA with an anti-single stranded DNA antibody.



Fluorescence in situ Hybridization (FISH)

Fluorescence in situ Hybridization (FISH) of Mouse Anti-5-mC antibody. Cells: metaphase chromosomes from HeLa cells. Treatment: colcemid (0.1 μ g/ml) for 1 - 2 hours. Fixation: ethanol/glacial acetic acid -20°C overnight then treated with 2N HCl for 30min at RT. Block: 0.1% Triton X and 1% BSA. Primary antibody: 5-mC antibody at 1:1000 for 1 hr at RT. Secondary antibody: goat anti-Mouse Alexa594 secondary antibody at 1:10,000 for 45 min at RT. Staining: 5-mC antibody as red signal (left), with blue DAPI (middle), and images merged (right).

**Dot Blot**

Dot Blot results of Mouse anti-5-mC monoclonal antibody.
Antigens: C control, mC, and hmC forms of the immunizing peptide. Load: 100 ng, 20 ng, and 4 ng as indicated.
Primary antibody: 5-mC antibody at 1:600 for 45 min at 4°C.
Secondary antibody: goat anti-mouse Dylight™488
secondary antibody at 1:10,000 for 45 min at RT. Block: 5% BLOTTO overnight at 4°C.

Disclaimer

This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information. All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation. All properties listed are typical characteristics and are not specifications. All suggestions and data are offered in good faith but without guarantee as conditions and methods of use of our products are beyond our control. All claims must be made within 30 days following the date of delivery. The prospective user must determine the suitability of our materials before adopting them on a commercial scale. Suggested uses of our products are not recommendations to use our products in violation of any patent or as a license under any patent of Rockland Immunochemicals, Inc. If you require a commercial license to use this material and do not have one, then return this material, unopened to: Rockland Inc., P.O. BOX 5199, Limerick, Pennsylvania, USA.