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## TECHNICAL DATASHEET

## Monoclonal Anti-5-METHYLCYTOSINE

Clone **33D3**

Ref # BIO.000.1

## Product description

Description	Monoclonal antibody to 5-methylcytosine
Host species	Mouse
Validated applications	ELISA, WB / IB, IP, FACS
Species reactivity	All species
Specificity	5-mC
Epitope	Modified base 5-methylcytidine found in DNA of plants and vertebrates

## Target exploration

### Overview:

5-methylcytosine (5-mC) is a modified base present in nucleic acids of plants and vertebrates. It was discovered in 1948 by Hotchkiss (\*). DNA methylation is a post-replicative process involved in the establishment of genomic imprinting, in the control of gene expression and of differentiation.

Carcinogenesis is associated with alterations of the DNA methylation pattern: a global hypomethylation is often detected in tumor tissues when compared to their normal counterpart. Simultaneously local hypermethylation sites are observed.

The monoclonal antibodies described here were raised against the modified ribonucleoside and they are specific for the presence of a methyl group on carbon 5 of the pyrimidine ring. These tools were used to discriminate between samples (tissues, cells, nuclei, chromosomes, biological fluids) obtained from healthy individuals and from cancer patients.

They can also provide insights on the distribution of methyl-rich regions of embryos after fertilization, on the methylation status of DNA in cells grown *in vitro*, either in situ or after extraction. Various protocols were developed allowing a wide range of techniques to be utilized (microscopy, flow cytometry, South/western blots, ELISA).

## Properties

Form	Liquid or lyophilized
Storage instructions	Store at +4°C short term (< 1 month). Aliquot and store at -20°C or -80°C. Avoid repeated freeze / thaw cycles
Storage buffer	Phosphate Buffer 10mM - NaCl 0.15M - pH 7,4
Concentration	1 mg.ml <sup>-1</sup>
Purity	Purified IgG fraction prepared by affinity chromatography on protein A
Isotype	IgG1,Lambda
Restrictions	For research use only.

## Applications

WB / IB	Suggested dilution: 1/250 (Southern blot 1/200)
ELISA	Suggested dilution: 1/10,000
MeDIP / CHIP	1-2 µg per IP
IF	Suggested dilution: 1/500
IHC	Use at an assay dependent dilution. Perform heat mediated antigen retrieval before commencing with IHC staining protocol
ICC	Use at concentration of 0.5-5 µg/ml
ChIP	Use at an assay dependent concentration
Flow cytometry	Use at an assay dependent dilution. (Use 10µl of working dilution to label 1,000,000 cells in 100µl. (see Habib, M. <i>et al.</i> (1999).)

Optimal dilutions/concentrations should be determined by the end user

## Publications

- Hotchkiss RD. The quantitative separation of purines, pyrimidines and nucleosides by paper chromatography. J Biol Chem. 1948;175:315-332.
- Lewis ZA, Honda S, Khlafallah TK, Jeffress JK, Freitag M, Mohn F, Schübeler D, and Selker EU (2009) Relics of repeat-induced point mutation direct heterochromatin formation in *Neurospora crassa* Genome Res 19: 427– 437.