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Monoclonal Antibody 5-Methylcytidine
BI-MECY-0100 • BI-MECY-0500 • BI-MECY-1000

Eurogentec products are sold for research or laboratory use only and are not to be administrated to humans or used for medical diagnostics.

Description
Monoclonal Antibody against 5-Methylcytidine

Form
Purified Ascites

Host
Mouse

Isotype
lgG1 / λ

[Ab]
1 mg / ml in PBS (+ 0.01 % thimerosal)

Specificity
5-Methylcytidine is a modified base found in DNA of plants and vertebrates. DNA methylation is a post-replication 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 DNA hypomethylation is often detected in tumor tissues, associated with local hypermethylation sites. This antibody has been developed to discriminate between the modified base and its normal counterpart. lt has been used to detect alterations in the urinary excretion of nucleosides by cancer patients, to visualize the distribution of methyl-rich regions along human chromosomes, to quantify *in situ* differences between normal and malignant cells from peripheral blood as well as on tissue sections.

Uses
This antibody is effective in ELISA, immunoblotting, cytochemistry, flowcytochemistry, immunohistochemistry and cytogenetics. The optimal working dilution should be determined for each specific assay condition.

Dilution

Blotting	1:500
Cytochemistry	1:500
lmmunohistochemistry	1:500
lmmunoprecipitation	1:50

Dot-Blot Assay with 5-Methylcytidine, Monoclonal Antibody, purified

– 2 mg of DNA was denatured in 0.4 M NaOH, 10 mM EDTA at 95°C for 10 min, and then neutralized by adding an equal volume of cold 2 M ammonium acetate (pH 7.0).

– Next, 2-fold dilutions of denatured DNA samples were spotted on a nitrocellulose membrane in an assembled Bio-Dot apparatus (Bio-Rad). Vacuum was subsequently applied to filter through DNA samples.

– The blotted membrane was washed with 2x SSC buffer, air-dried and vacuum-baked at 80°C for 2 hrs.

– The membrane was then blocked with 5% non-fat milk and incubated with monoclonal 5meC antibody (1:1000)

– Binding of an HRP-conjugated secondary antibody (1:12000) was visualized by enhanced chemiluminescence (ECL).

– To ensure equal spotting of total DNA on the membrane, the same blot was then stained with 0.02% methylene blue in 0.3 M sodium acetate (pH 5.2).

Modified from:
Huang Y, Pastor WA, Shen Y, Tahiliani M, Liu DR, et al. (2010) The Behaviour of 5-Hydroxymethylcytosine in Bisulfite Sequencing. PLoS ONE 5(1): e8888. doi:10.1371/journal.pone.0008888

Storage
Store at -20 °C (-80 °C for long term storage). lt is suggested that the total volume be divided into usable aliquots upon initial thaw.

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Restriction

For research use only, not for human or *in-vivo* use

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DNA global hypomethylation in EBV-transformed interphase nuclei. Fxp. Cel/ Res. 249 : 46-53, 1999.
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