Product datasheet

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

Overview

Product name  Anti-5-methylcytosine (5-mC) antibody [33D3]
Description  Mouse monoclonal [33D3] to 5-methylcytosine (5-mC)
Host species  Mouse
Specificity  Clone 33D3 has been developed to discriminate between the modified base 5-MeCyd and the normal counterpart cytosine.

Tested applications  Suitable for: IHC-P, MeDIP, ChiP, IP, Southern Blot, Dot blot, Flow Cyt, IHC-Fr

Unsuitable for: ICC

Species reactivity  Reacts with: Species independent

Immunogen  Chemical/ Small Molecule corresponding to 5-methylcytosine (5-mC).

General notes  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.

Properties

Form  Liquid

Storage instructions  Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle.

Storage buffer  pH: 7.4
Preservative: 0.01% Thimerosal (merthiolate)
Constituent: 99.99% PBS

Purity  Protein A purified

Primary antibody notes  Ab10805 recognises the modified base 5-methylcytidine found in DNA of plants and vertebrates. DNA methylation is a DNA modification process, which is involved in the control of gene expression. Reports suggest that in tumours, DNA is frequently globally hypomethylated compared to the DNA from normal tissue.

Clonality  Monoclonal

Clone number  33D3
Myeloma: Sp2/0-Ag14  
Isotype: IgG1  
Light chain type: kappa

Applications

Our Abpromise guarantee covers the use of ab10805 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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<tbody>
<tr>
<td>IHC-P</td>
<td>★★★★★</td>
<td>Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.</td>
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<tr>
<td>MeDIP</td>
<td></td>
<td>Use at an assay dependent concentration. PubMed: 17845077 Used 5ul in 200ul reaction with 2ug digested genomic DNA from Drosophila.</td>
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<tr>
<td>ChIP</td>
<td>★★★★★</td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>IP</td>
<td>★★★★★</td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>Southern Blot</td>
<td></td>
<td>1/200.</td>
</tr>
<tr>
<td>Dot blot</td>
<td></td>
<td>Use at an assay dependent concentration. PubMed: 24386123</td>
</tr>
<tr>
<td>Flow Cyt</td>
<td>★★★★★</td>
<td>Use at an assay dependent concentration. 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.</td>
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<tr>
<td>IHC-Fr</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
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Application notes

Is unsuitable for ICC.

Target

Form

The native context of double-stranded DNA may obstruct antibody binding to 5-methylcytosine. For successful detection of 5-methylcytosine, we recommend that the DNA is denatured to make the nucleotides accessible for the antibody. Denaturing methods vary depending on each application.

Images
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-5-methylcytosine (5-mC) antibody [33D3] (ab10805)
This image is courtesy of an Abreview submitted by Ms Sara Maj Wätjen Hyldig

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.

Native ChIP analysis using unpurified ab10805 binding 5-methylcytosine (5-mC) in nuclear cell lysate derived from mouse embryonic stem cells. Samples were incubated with primary antibody (2µg/µg chromatin) for 16 hours at 4°C in a Glycerol IP buffer. Protein binding was detected using real-time PCR.

Positive control: Magoh2 in HMT KO cells.
Negative Control: Magoh2 in WT cells.
Dot blot carried out using ab10805 (left blot), showing specificity to 5-methylcytosine. Indicated amounts (20–5 ng) of oligonucleotides containing either 5mC (50%) or 5hmC (20%) were spotted on positive charged nylon membrane and detected either with 5mC (ab10805) or 5hmC antibody.

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