5-methylcytosine (5-mC) monoclonal antibody for ICC/IF - Classic

Cat. No. C15200003

Type: Monoclonal	Specificity: Human, mouse, drosophila, other (wide range): positive.
Size: 50 µg/39 µl	Isotype: IgG1
Concentration: 1.3 µg/µl	Host: Mouse
Lot No.: 003	Purity: Protein A purified monoclonal antibody.
Storage buffer: PBS containing 0.05% azide.	Storage conditions: Store at -20°C; for long storage, store at -80°C. Avoid multiple freeze-thaw cycles.
Precautions: This product is for research use only. Not for use in diagnostic or therapeutic procedures.	

Last Data Sheet Update: July 30, 2020

Description

Monoclonal antibody raised in mouse against 5-mC (5-methylcytosine) conjugated to BSA.

Applications

Applications	Suggested dilution	References
MeDIP *	1-2 µg per IP	
Dot Blotting **	1:600	Fig 1
Immunofluorescence	1:1,000	Fig 2, 3
FISH	1:200 - 1:1,000	Fig 4, 5

* Please note that the optimal antibody amount per IP should be determined by the end-user. We recommend testing 1-5 µg per IP.

** Dot blot was only performed to demonstrate the specificity. This antibody is not recommended for dot blot on biological samples

Target Description

Monoclonal antibody raised in mouse against 5-mC (5-methyl Cytidine) conjugated to BSA.

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Validation Data

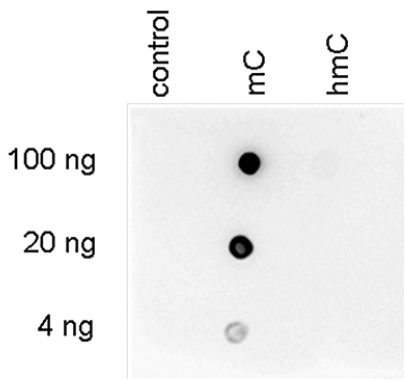

Figure 1. Dot blot analysis using the Diagenode monoclonal antibody directed against 5-mC

To demonstrate the specificity of the Diagenode antibody against 5-mC (Cat. No. C15200003), a Dot blot analysis was performed using the hmC, mC and C controls from the Diagenode "5-hmC, 5-mC & cytosine DNA Standard Pack" (Cat. No. AF-101-0002). One hundred to 4 ng (equivalent of 5 to 0.2 pmol of C-bases) of the controls were spotted on a membrane (Amersham Hybond-N+). The antibody was used at a dilution of 1:600. Figure 1 shows a high specificity of the antibody for the methylated control.

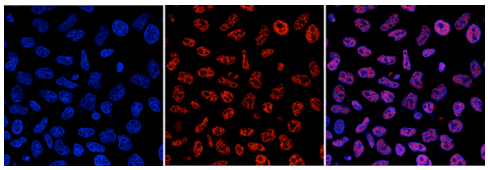

Figure 2. Immunofluorescence results obtained with the Diagenode monoclonal antibody directed against 5-mC

HeLa cells were stained with the Diagenode antibody against 5-mC (Cat. No. C15200003) and with DAPI. Cells were fixed with 4% formaldehyde for 10' and blocked with PBS/TX-100 containing 1% BSA. The cells were immunofluorescently labelled with the 5-mC antibody (middle) diluted 1:1,000 in blocking solution followed by an anti-mouse antibody conjugated to Alexa594. The left panel shows staining of the nuclei with DAPI. A merge of the two stainings is shown on the right.

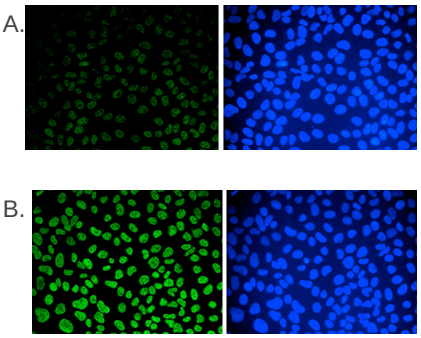

Figure 3. Immunofluorescence results obtained with the Diagenode monoclonal antibody directed against 5-mC

Human osteosarcoma (U2OS) cells were stained with the Diagenode monoclonal antibody against 5-mC (Cat. No. C15200003). Cells were fixed with 2.5% PFA in PBS for 30', permeabilised with 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. After blocking with PBS containing 0.1% Triton X-100 and 1% BSA, the cells were immunofluorescently labelled with the 5-mC antibody diluted 1:500 in blocking solution, followed by a goat anti-mouse antibody conjugated to Alexa488. Figure 3A: cells were immunofluorescently labelled with the 5-mC antibody after incubation of the antibody with 50 µM mCTP (left) or with DAPI (right). Figure 3C: staining of the cells with the 5-mC antibody after incubation of the antibody with 50 µM hmCTP and with DAPI.

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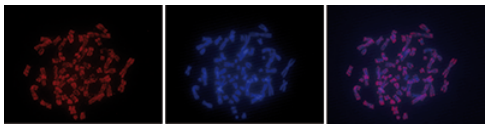

Figure 4. FISH using the Diagenode monoclonal antibody directed against 5-mC

To detect methylated chromosomal regions, FISH was performed on metaphase chromosomes from HeLa cells using the Diagenode monoclonal antibody against 5-mC (Cat. No. C15200003). The cells were blocked in metaphase by treatment with colcemid (0.1 µg/ml) for 1 - 2 hours, fixed overnight at -20°C with ethanol/glacial acetic acid and treated with 2N HCl for 30' at room temperature. Subsequently, the cells were blocked with PBS containing 1% BSA and 0.1% Triton X-100 and stained with the 5-mC antibody (left) diluted 1:1,000 in blocking solution, followed by an anti-mouse antibody conjugated to Alexa594. The middle panel shows staining of the chromosomes with DAPI. A merge of the two stainings is shown on the right.

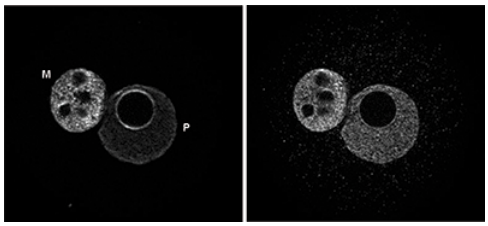

Figure 5. FISH using the Diagenode monoclonal antibody directed against 5-mC

To detect methylated chromosomal regions, FISH was performed on metaphase chromosomes from HeLa cells using the Diagenode monoclonal antibody against 5-mC (Cat. No. C15200003). The cells were blocked in metaphase by treatment with colcemid (0.1 µg/ml) for 1 - 2 hours, fixed overnight at -20°C with ethanol/glacial acetic acid and treated with 2N HCl for 30' at room temperature. Subsequently, the cells were blocked with PBS containing 1% BSA and 0.1% Triton X-100 and stained with the 5-mC antibody (left) diluted 1:1,000 in blocking solution, followed by an anti-mouse antibody conjugated to Alexa594. The middle panel shows staining of the chromosomes with DAPI. A merge of the two stainings is shown on the right.

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