TECHNICAL DATASHEET

LOT: 002

HOLOGIC® Diagenode

5-hydroxymethylcytosine (5-hmC) antibody (mouse)

Cat. No. C15200200

Lot:	002
Size:	20 µg / 50 µg / 100 µg
Type:	Monoclonal | **hMeDIP grade**
Isotype:	IgG1κ
Source:	Mouse
Concentration:	2.07 µg/µl

Specificity:	Human, mouse, other (wide range): positive
Purity:	Protein A purified monoclonal antibody
Storage buffer:	PBS containing 0.05% azide

Storage: 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.

Description: Monoclonal antibody raised in mouse against 5-hydroxymethylcytosine conjugated to BSA.

Applications

Applications	Suggested dilution	References
hMeDIP*	2 µg per IP	Fig 1
Dot blotting	2 µg/ml	Fig 2
ELISA	1:500	Fig 3

*Please note that the optimal antibody amount per IP should be determined by the end-user. We recommend testing 0.5-5 µg per IP.

Diagenode, SA. BELGIUM | EUROPE
LIEGE SCIENCE PARK
Rue du Bois Saint-Jean, 3
4102 Seraing - Belgium
Tel: +32 4 364 20 50
Fax: +32 4 364 20 51
orders.diagenode@hologic.com
support.diagenode@hologic.com

Diagenode, LLC. USA | NORTH AMERICA
400 Morris Avenue, Suite 101
Denville, NJ 07834 - USA
Tel: +1 862 209-4680
Fax: +1 862 209-4681
orders.na@diagenode.com
info.na@diagenode.com

Last update: February, 2024

Results

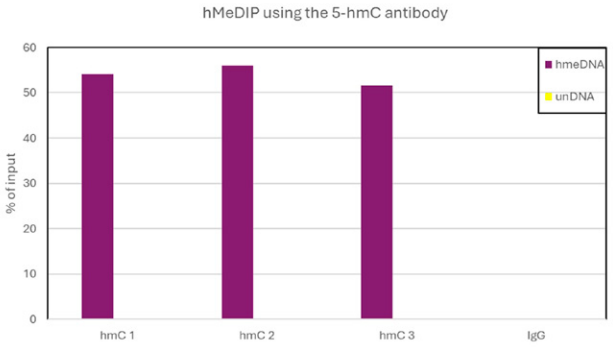

Figure 1: hMeDIP results obtained with the monoclonal antibody directed against 5-hmC

hMeDIP (hydroxyMethylated DNA immunoprecipitation) was performed in triplicate on 1 µg fragmented human genomic DNA using 2 µg of the monoclonal antibody against 5-hmC (cat. No. C15200200) and the hMeDIP Kit (cat. No. C02010031). IgG (2 µg) was used a negative IP control. The fragmented DNA was spiked with the internal controls present in the kit (hydroxymethylated DNA (hmeDNA) as a positive and unmethylated DNA (unDNA) as a negative control) prior to performing the IP.
QPCR was performed with optimized primer sets, included in the kit, specific for the hydroxymethylated and unmethylated DNA controls. Figure 1 shows the recovery expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

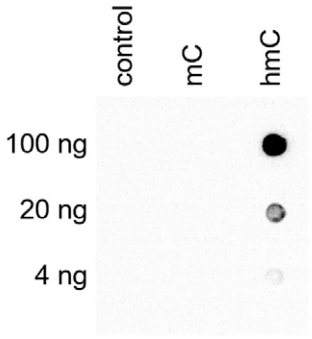

Figure 2: Dot blot analysis using the monoclonal antibody directed against 5-hmC

To demonstrate the specificity of the antibody against 5-hmC (cat. No. C15200200), a Dot blot analysis was performed using an unmodified, a 5-mC and a 5-hmC containing DNA fragment. 100 to 4 ng (equivalent of 5 to 0.2 pmol of C-bases) of the DNA fragments were spotted on a membrane. The antibody was used at a concentration of 2 µg/ml. Figure 2 shows a high specificity of the antibody for the hydroxymethylated fragment.

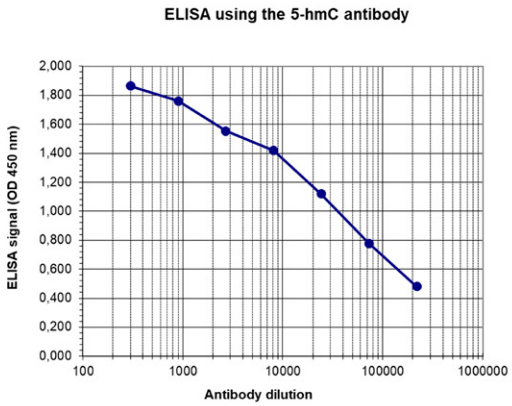

Figure 3: Determination of the antibody titer

To determine the titer, an ELISA was performed using a serial dilution of the mouse monoclonal antibody directed against 5-hmC (Cat No. C15200200) in antigen coated wells. The antigen used was the 5-hmC base coupled to KLH. By plotting the absorbance against the antibody dilution, the titer of the antibody was estimated to be 1:40,000.