

Product no **AS16 3166****Anti-5-hmC | 5-hydroxymethylcytosine (polyclonal)****Product information**

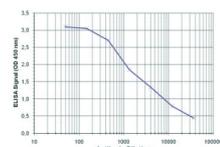
Immunogen	BSA-conjugated molecule: 5-hydroxymethylcytosine (5-hmC)
Host	Rabbit
Clonality	Polyclonal
Purity	Protein G purified.
Format	Liquid
Quantity	100 µl
Storage	Store lyophilized/reconstituted at -20°C; for long term storage Store at -80°C; once reconstituted make aliquots to avoid repeated freeze-thaw cycles. Please remember to spin the tubes briefly prior to opening them to avoid any losses that might occur from material adhering to the cap or sides of the tube.

Additional information | This serum contains 0,05 % sodium azide**Application information**

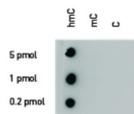
Recommended dilution	1 : 200 (Dot), 1 : 500 (ELISA), 2,5 µl/IP (hMeDIP)
Confirmed reactivity	Human
Predicted reactivity	Mouse, broad species range
Not reactive in	No confirmed exceptions from predicted reactivity are currently known

application example

An **hydroxymethylated DNA IP (hMeDIP)** was performed using the rabbit polyclonal antibody directed against 5-hydroxymethylcytosine. The IgG isotype antibodies from rabbit were used as negative control. The DNA was prepared with the GenDNA module of the hMeDIP kit and sonicated with our Bioruptor® to have DNA fragments of 300-500 bp. 1 µg of human HeLa cells DNA were spiked with non-methylated, methylated, and hydroxymethylated fragments. The IP'd material has been analysed by qPCR using the primer pair specific for the 3 different control sequences. The obtained results show that the rabbit polyclonal for 5-hmC is highly specific for this base modification (no IP with non-methylated or methylated C bases containing fragments).



ELISA: to determine the titer, an ELISA was performed using a serial dilution of the rabbit polyclonal antibody directed against 5-hmC in antigen coated wells. The antigen used was BSA coupled to the 5-hmC base. By plotting the absorbance against the antibody dilution, the titer of the antibody was estimated to be 1: 3 500.



Dot blot analysis of the 5-hmC rabbit polyclonal antibody with the C, mC and hmC PCR controls 100 to 4 ng (equivalent of 5 to 0.2 pmol of C-bases) of the hmC, mC and C PCR controls from the "5-hmC, 5-mC & cytosine DNA Standard Pack" were spotted on a membrane (Amersham Hybond-N+). The membrane was incubated with the rabbit 5-hydroxymethylcytosine polyclonal antibody (dilution 1:200). The membranes were exposed for 30 seconds.