

# Agrisera

This product is for research use only (not for diagnostic or therapeutic use)

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Product no **AS16 3166**

## 5-hmC | 5-hydroxymethylcytosine (polyclonal)

### Product information

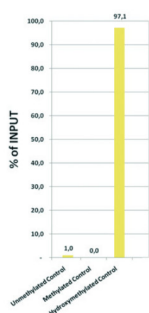
<b>Background</b>	<b>5-hmC   5-hydroxymethylcytosine</b> is a recently discovered DNA modification which results from the enzymatic conversion of 5-methylcytosine into 5-hydroxymethylcytosine by the TET family of oxygenases. It may have an important roles distinct from that of 5-methylcytosine (5-mC). 5-hmC bases have been identified in Purkinje neurons, in granule cells and embryonic stem cells where they are present at high levels (up to 0,6% of total nucleotides in Purkinje cells).
<b>Immunogen</b>	BSA-conjugated molecule: 5-hydroxymethylcytosine (5-hmC)
<b>Host</b>	Rabbit
<b>Clonality</b>	Polyclonal
<b>Purity</b>	Protein G purified
<b>Format</b>	Liquid
<b>Quantity</b>	100 µl
<b>Storage</b>	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 tubes briefly prior to opening them to avoid any losses that might occur from lyophilized material adhering to the cap or sides of the tubes.
<b>Tested applications</b>	Dot blot (Dot), ELISA (ELISA), hMeDIP (hydroxymethylated DNA Immunoprecipitation (IP))
<b>Related products</b>	<a href="#">AS16 3164</a>   Anti-5-hmC   5-hydroxymethylcytosine (monoclonal), mouse antibodies <a href="#">collection of antibodies to epigenetics</a>
<b>Additional information</b>	This serum contains 0.05 % sodium azide.

### Application information

<b>Recommended dilution</b>	1 : 200 (Dot), 1 : 500 (ELISA), 2.5 µl/IP (hMeDIP)
<b>Confirmed reactivity</b>	Human
<b>Predicted reactivity</b>	Mouse, broad species range
<b>Not reactive in</b>	No confirmed exceptions from predicted reactivity are currently known.

For high resolution images, please visit the specific product page at [www.agrisera.com](http://www.agrisera.com)

#### application example



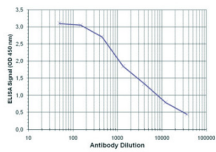
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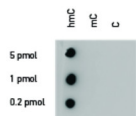
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An **hydroxymethylated DNA IP (hMeDIP)** was performed using the rabbit polyclonal antibody directed against 5-hydroxymethylcytosine. The IgG isotype antibodies from rabbit were used as negative control. The DNA was prepared with the GenDNA module 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.