

# HIGH THROUGHPUT & RAPID hMeDIP

The EpiQuik™ Hydroxymethylated DNA Immunoprecipitation (hMeDIP) Kit is a complete set of optimized buffers and reagents that allows the experimenter to selectively enrich DNA fragments containing 5-hydroxymethylcytosine in a convenient 96-well plate format. The entire procedure can be finished within 3 hours, producing enriched hydroxymethylated DNA suitable for standardized DNA detection methods including PCR (hMeDIP-PCR) and microarrays (hMeDIP-chip).

### About Hydroxymethylated DNA Immunoprecipitation

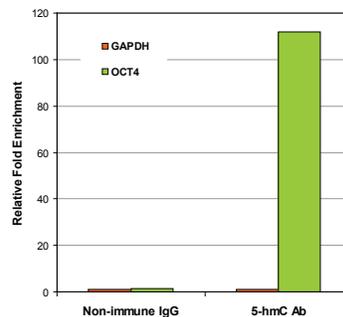
hMeDIP is a genome-wide, large scale technique to enrich hydroxymethylated DNA by isolating hydroxymethylated DNA fragments with a 5-hydroxymethylcytosine antibody. With hMeDIP, the immunoprecipitated fractions of hydroxymethylated DNA can be determined by PCR to evaluate the hydroxymethylation state of individual regions or by microarray for hydroxymethylome-level analysis on a genome-wide scale.

### Taking the Epigentek Approach

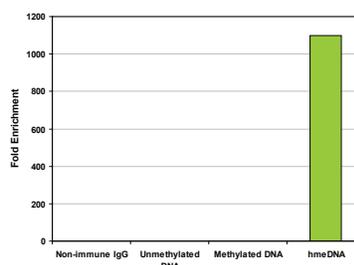
Epigentek has developed an innovative method to capture DNA fragments containing 5-hmC, and incorporated this method into its EpiQuik™ Hydroxymethylated DNA Immunoprecipitation (hMeDIP) Kit. This kit uses a high affinity 5-hmC antibody to selectively capture double-stranded or single stranded DNA fragments containing 5-hmC. The kit has the following features:

- ✓ Extremely fast and convenient protocol with a total procedure time (from input sample to ready-to-use hydroxymethylated DNA) of less than 3 hours, which includes a minimal handling time of less than 20 minutes.
- ✓ Flexible 96 stripwell microplate format makes the assay very easy to handle: manual method with one reaction at a time or high throughput method with 96 reactions at a time.
- ✓ Highly efficient enrichment: ratio of positive/negative control > 1000.
- ✓ Low DNA input requirement of as low as 0.1 µg per reaction.
- ✓ High reproducibility through pre-optimized hMeDIP conditions.
- ✓ Compatible with various downstream analysis workflows including hMeDIP-PCR and hMeDIP-chip

- 1 DNA shearing
- 2 hmeDNA/antibody immunoprecipitation
- 3 Release of hmeDNA from hmeDNA/antibody complex
- 4 Elution of hmeDNA
- 5 PCR, microarray, or similar downstream application



**Fig. 1 | Sensitive detection of gene-specific hydroxymethylation by hMeDIP-qPCR.** Human brain DNA (500 ng) was fragmented to 200-600 bps with EpiSonic 1000 (Cat. No. EQC-1000). The fragmented DNA was used for hydroxymethylated DNA enrichment with the EpiQuik™ hMeDIP Kit. Eluted DNA was analyzed by real time PCR with primers specific for OCT4 or GAPDH sequences in the promoter regions. Results show that the promoter region is hydroxymethylated in OCT4 but not in GAPDH. Fold-enrichment represents the amount of recovered DNA and was calculated based on the Cts.



**Fig. 2 | Selective enrichment of hydroxymethylated DNA with the EpiQuik™ hMeDIP Kit.** 50 pg of unmethylated, methylated, and hydroxymethylated DNA control were each spiked into fragmented human genomic DNA (500 ng). hMeDIP was processed with the 5-hmC antibody and non-immune IgG included in the kit. Eluted DNA was analyzed by real time PCR with the control primers included in the kit to detect the presence of spiked control DNA. Fold-enrichment represents the amount of recovered control DNA and was calculated based on the Cts.

	EpiQuik	Supplier 1	Supplier 2
Working Principle	Immuno-capture	Immuno-capture	Immuno-capture
Working Format	Microplate-based	Beads-based	Beads-based
Max Number of Reactions Per Run	96	10	16
High Throughput Capable	Yes	No	No
Convenience (Assay Steps)	12 steps	20 steps	26 steps
Total Procedure Time	< 3 hours	~24 hours	~24 hours
Physical Handling Time	< 15 minutes	> 1 hour	> 1 hour
Amount of Input DNA	0.1-1 µg ss/dsDNA	0.1-1 µg ss/dsDNA	1.2 µg ss/dsDNA
Fold Enrichment w/Spiked 5-hmC Control	>1000	>1000	>1000
Fold Enrichment w/Genomic DNA Samples From Cells/Tissues	>100	N/A	N/A
Yield of Enriched hmeDNA from Genomic DNA Samples	~4 ng per 500 ng of human brain DNA	N/A	N/A
Specificity (Positive/Negative Ratio)	>99%	>99%	>99%
Cost Per Reaction	\$10	\$35	\$47

Product Name	Size	Cat. No.
EpiQuik Hydroxymethylated DNA Immunoprecipitation (hMeDIP) Kit	24 reactions	P-1038-24
	48 reactions	P-1038-48
	96 reactions	P-1038-96