abcam

Product datasheet

MeDIP Ultra Kit ab185904

画像数 2

製品の概要

製品名	MeDIP Ultra Kit
サンプルの種類	DNA
全工程の試験時間	3h 00m
製品の概要	The MeDIP Ultra Kit is a complete set of optimized reagents to enrich and capture methylated DNA fragments in a convenient microplate-based format. The method, methylated DNA immunoprecipitation (MeDIP), uses a monoclonal antibody specific to 5-methylcytosine to immunoprecipitate methylated genomic DNA. The enriched methylated fractions can then be used for gene-specific DNA methylation analysis on a genome wide scale. The highly sensitive and specific format of the kit can use DNA isolated from various species. The methylated DNA that is enriched with this kit can be used for various downstream applications including qualitative and quantitative PCR (MeDIP-PCR), microarray (MeDIP-chip) and especially sequencing (MeDIP-seq). **Starting Materials, Input Amount, & Expected Yield** The starting material should be good quality purified DNA. The amount of DNA for each reaction can be 50 ng (approximately 10,000 cells) to 500 ng. For an optimal reaction, the input DNA amount should be 100-200 ng per well. The yielded methylated DNA is about 4 ng for 100 ng input DNA (4%), which is consistent with the expected percentage (4-5%) at which the highest sensitivity and specificity for enriched methylated DNA has been demonstrated by bisulfite sequencing.
特記事項	Core mechanisms for epigenetic alteration of genomic DNA are hypermethylation of CpG islands in specific genes and global DNA hypomethylation. Region-specific DNA methylation plays an important role in the repression of gene transcription and is mainly found in 5'-CpG-3'dinucleotides within promoters or in the first exon of genes. Global DNA hypomethylation is likely caused by methyl-deficiency due to a variety of environmental influences. It has been demonstrated that alterations in DNA methylation are associated with many diseases, especially cancer. Highly specific isolation of methylated DNA combined with next generation sequencing for genome-wide methylation analysis should provide an advantage for convenient and comprehensive identification of methylation status of normal and diseased cells, such as cancer cells. Such analysis requires the isolated methylated DNA to contain minimal background in order to achieve high specificity (>98%) for reliably identifying true methylated regions. The major method for enriching methylated DNA used for genome-wide methylation profiling is methylated DNA immunoprecipitation (MeDIP). However, currently used MeDIP methods, represented by most commercially available kits, have significant weaknesses including highly non-specific

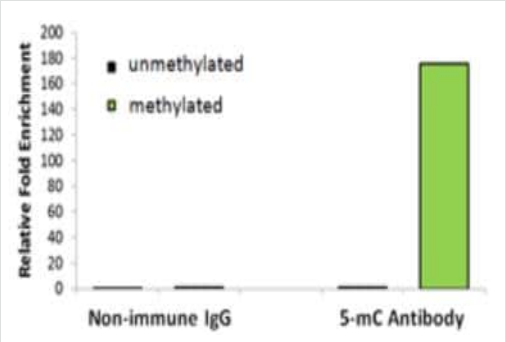
enrichment (amount of enriched DNA is >75% of the amount of input DNA), time consuming, labor intensive, and has low throughput. Thus, for effectively and specifically capturing methylated DNA used for next generation sequencing analysis, an ideal MeDIP method requires maximum sensitivity with minimal background levels. TheMeDIP Ultra Kit is designed to achieve these goals by maximizing sensitivity and minimizing non-specific background signals, and is a significant improvement over previous MeDIP kits.

製品の特性

保存方法 Please refer to protocols.

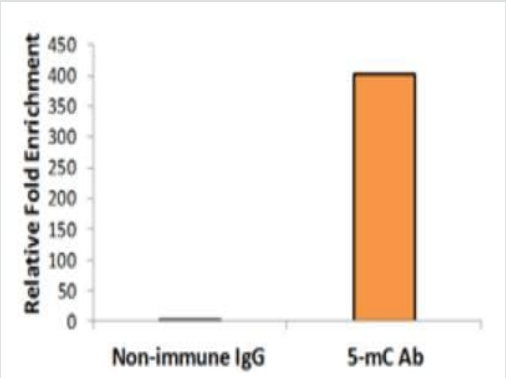
内容	24 tests	48 tests
5-mC Antibody	1 x 25µl	1 x 50µl
8-Well Assay Strips (with Frame)	1 x 3 units	1 x 6 units
8-Well Strip Caps	1 x 3 units	1 x 6 units
Adhesive 8-Well Strip Film	1 x 3 units	1 x 6 units
Blocker Solution	1 x 200µl	1 x 400µl
Control mDNA (200 ng/ml)	1 x 5µl	1 x 10µl
Control Primer F	1 x 5µl	1 x 10µl
Control Primer R	1 x 5µl	1 x 10µl
Control unDNA (200 ng/ml)	1 x 5µl	1 x 10µl
DNA Binding Solution	1 x 7ml	1 x 14ml
DNA Elution Buffer	1 x 1ml	1 x 2ml
DNA Release Buffer	1 x 14ml	1 x 28ml
F-Collection Tube	1 x 30 units	1 x 50 units
F-Spin Column	1 x 30 units	1 x 50 units
MeDIP Buffer	1 x 4ml	1 x 8ml
Non-Immune IgG (1 mg/ml)	1 x 10µl	1 x 20µl
Proteinase K (10 mg/mL)	1 x 28µl	1 x 56µl
Wash Buffer	1 x 15ml	1 x 30ml

画像



Selective enrichment of methylated DNA.

50 pg of unmethylated or methylated DNA control were each spiked into fragmented human genomic DNA (100 ng). MeDIP was processed with the included 5-mC monoclonal 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 real time PCR Ct value.



Sensitive detection of gene-specific methylation.

Fully methylated HeLa DNA (500 ng) was fragmented to 100-500 bps. The fragmented DNA was used for methylated DNA enrichment with ab185904. Eluted DNA was analyzed by real time PCR with primers specific for MLH1 sequences in the promoter regions. Results show the specificity of the 5-mC antibody and low background for non-immune IgG. Fold-enrichment represents the amount of recovered DNA and was calculated based on the real time PCR Ct value.

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