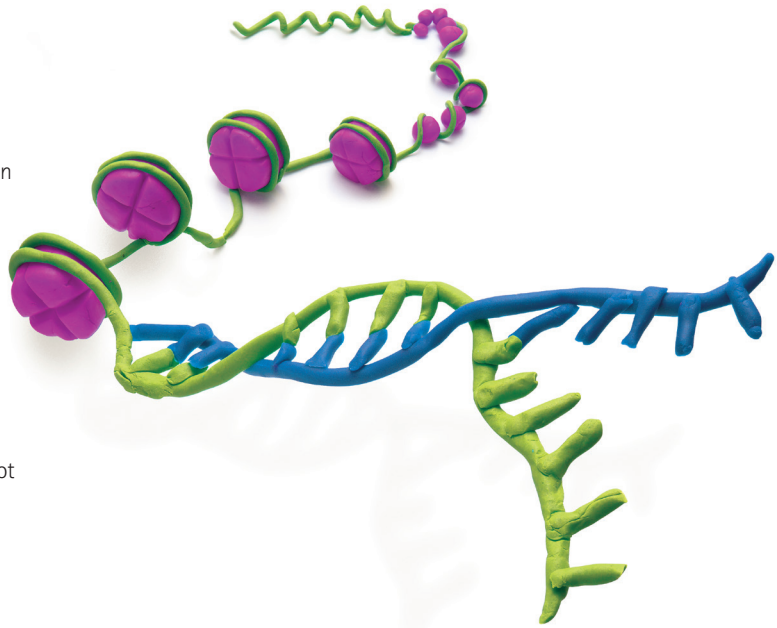
Shaping Epigenetics Discovery

Kits, Assays, Controls, Inhibitors, and Antibodies for DNA Methylation

With the expertise of Calbiochem®, Chemicon®, and Upstate®

DNA methylation is an important epigenetic mechanism regulating gene silencing, imprinting, embryonic development, and chromosome stability. DNA methylation occurs on the 5 carbon position of cytosine residues mainly within CpG dinucleotides to form 5-methylcytosines (5-mC). The reaction is catalyzed by DNA methyltransferases (DNMTs). 5-methylcytosines residues may also be hydroxylated by TET enzymes to form 5-hydroxymethylcytosine (5-hmC), which has differing roles from 5-mC. EMD Millipore provides robust tools that enable you to not only detect and quantify 5-mC and 5-hmC, but also to accurately distinguish between these modifications.



For more information, visit:

www.emdmillipore.com/epigenetics

Innovative products to analyze DNA methylation marks

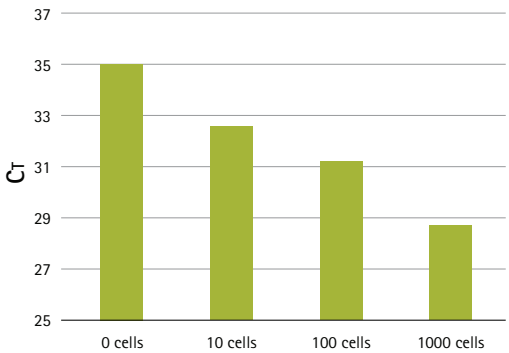
- **CpGenome™ Direct Prep kits –** bisulfite conversion directly from cells, blood, FFPE or tissue. Single tube and high throughput options.
- **CpGenome™ Turbo Bisulfite Kits** – efficient bisulfite conversion with 1 µg to 1 ng of input DNA
- **CpG MethylQuest™ Kits** – easy MBD2b-mediated pulldown of methylated DNA
- **CpG WIZ® Kits** – well-designed primers for amplification of methylated DNA
- **CpGenome™ DNA Standards** – methylated, hydroxymethylated, or unmethylated DNA for essential negative and positive controls
- **Antibodies for MeDIP and hMeDIP** – high specificity and affinity, validated in various applications
- **Inhibitors** – selective and potent inhibition of enzymes involved in DNA methylation
- **Enzymes and Recombinant Proteins –** highly purified and active proteins for DNA methylation assays

EMD Millipore is a division of Merck KGaA, Darmstadt, Germany

CpGenome™ Direct Prep Bisulfite Modification Kit:

Bisulfite conversion directly from cultured cells, blood, fresh or fixed tissue

(Catalogue No. 17-10451, 17-10452, 17-10454)

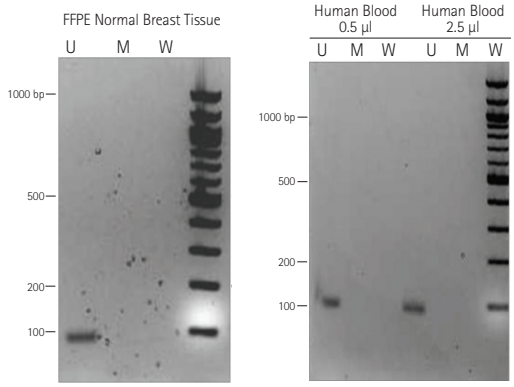


Effective bisulfite conversion from as few as 10 cells

Following the CpGenome™ Direct Prep protocol, 0, 10, 100 and 1000 HeLa cells were bisulfite-converted followed by real-time quantitative PCR detection with a CpG WIZ® BRCA1 U primer.

More easily perform DNA methylation analysis

- Direct bisulfite conversion of cells, tissues, blood and FFPE samples
- Conversion efficiencies >99.5%
- Bisulfite conversion from as few as 10 cells or as low as 50 pg of input DNA
- Fast, simple, and streamlined protocol for one-step bisulfite conversion
- In-column desulfonation allows recovery of DNA without additional precipitation steps for more consistent results
- Suitable for downstream analysis by methylation-specific PCR, restriction digestion, sequencing, microarray hybridization, etc.
- Available in 96 well plate format



Direct bisulfite conversion of FFPE samples (left) and blood samples (right).

A normal human FFPE sample and human blood samples were directly treated with proteinase K and bisulfite-converted as described in the CpGenome™ Direct Prep protocol. After conversion, the CpG WIZ® BRCA1 Amplification Kit was used to amplify converted DNA using methylation-specific PCR. Amplified products were analyzed on a 2% agarose gel.

U: CpG WIZ ® Unmethylated Primer

M: CpG WIZ® Methylated Primer

W: CpG WIZ® Wild Type Primer

Rapid and Efficient Bisulfite Conversion:

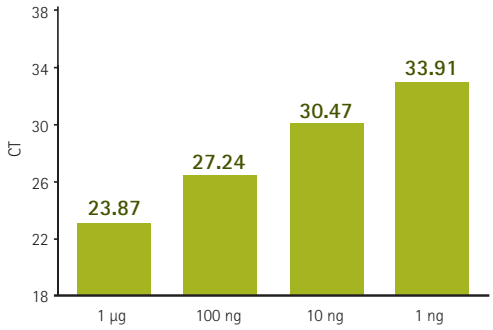
CpGenome™ Turbo Bisulfite Modification Kit

(Catalogue No. S7847)

Complete conversion of unmethylated cytosines in 90 minutes!

- Conversion efficiencies of 99.9% without over-conversion
- Optimized protocol enables virtually complete conversion of input samples with minimal DNA damage
- Accommodates 1 µg to 1 ng input sample
- Recover modified DNA in as little as 25 µL final volume
- Spin-column-based desulfonation and isolation procedure promotes efficient recovery of modified DNA for various downstream analyses

Reliable performance across a range of input sample amounts



Sensitive and Reliable Bisulfite Conversion from 1 ng to 1 µg Methylated DNA (Catalogue No. S7821) was bisulfite treated as described in the CpGenome™ Turbo protocol and eluted in 50 µL (1 µg and 100 ng samples) or in 25 µL (10 ng and 1 ng samples). Conversion was evaluated by quantitative PCR using the CpG WIZ® MGMT methylated primer set (Catalogue No. S7803).

Specific Enrichment of Methylated DNA:

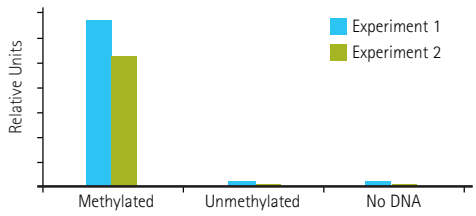
CpG MethylQuest™ DNA Isolation Kit

(Catalogue No. 17-10035)

Featuring a high affinity recombinant methyl-binding domain (MBD) protein pre-bound to magnetic beads, a rapid protocol, and a simple elution procedure that yields methylated DNA ready for downstream analysis, this high affinity kit enriches for methylated DNA from as little as 1 ng of DNA!

- Reliable performance from 1 ng to 1 µg of DNA
- GST-MBD2b capture protein pre-bound to magnetic beads for consistent results
- No detectable binding of unmethylated or hemimethylated regions
- Simple and fast 2-hour magnetic bead-based protocol
- Elute ready-to-use DNA; avoid additional cleanup steps that reduce yields

Specific binding of methylated DNA



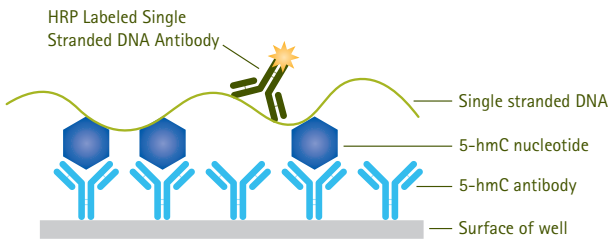
CpG MethylQuest™ MBD protein binds methylated DNA, but not unmethylated DNA. CpG MethylQuest™ protein was incubated with fully methylated or unmethylated p16 amplicons immobilized on magnetic beads, or a no-DNA control. Beads were washed and CpG MethylQuest™ protein was detected with an anti-GST antibody-horseradish peroxidase conjugate). The bars represent duplicate experiments.

Analysis of 5-methylcytosine (5-hmC)

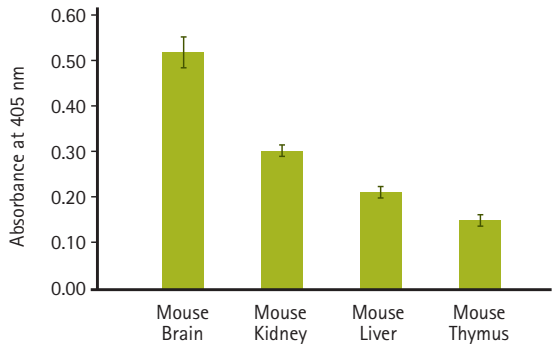
17-10091 CpGenome™ 5-hmC Quantitation Kit

CpGenome™ 5-hmC Quantitation Kit

(Catalogue No. 17-10091)



Simple ELISA-based Assay Procedure Reliable assay work flow allows for sensitive detection of 5-hmC in genomic DNA samples



Tissue-Specific Detection of 5-hmC Levels 100ng of genomic DNA from different tissues (CpGenome™ Mouse 5-mC and 5-hmC DNA Standards, Catalogue No. S8004) was analyzed using the CpGenome 5-hmC Quantitation kit. Data demonstrates reliable detection of differences in 5-hmC levels of input samples consistent with those previously determined by LC/MS analysis (mouse brain 0.55%; mouse kidney 0.23% mouse liver 0.11%; mouse thymus 0.03%)

The CpGenome™ 5-hmC Quantitation Kit allows determination of absolute and relative amounts of 5-hydroxymethylcytosine (5-hmC) in genomic DNA samples. This assay utilizes a rapid, streamlined, automation-compatible, ELISA-based protocol for sensitive quantification of 5-hmC DNA obtained from cultured cells or tissues from a wide variety or species

- Highly specific detection of 5-hmC DNA
- Limit of quantitation down to 0.03% of input genomic DNA
- Evaluate 5-hmC levels using 25 ng to 200 ng of input DNA per well
- Streamlined, simple, automation-friendly ELISA-based protocol with colorimetric detection
- Includes set of 5-hmC of standards for precise quantitation
- Ideal for global and tissue-specific detection/ quantitation, as well as high-throughput screening of 5-hmC activators and inhibitors

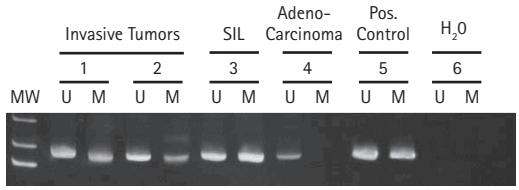
Easy Amplification of Methylated DNA

Methylation-specific PCR (MSP) is an established technique for mapping and monitoring methylation patterns in CpG islands.

EMD Millipore's CpG WIZ® kits simplify MSP:

- Sensitive detection of methylated and unmethylated DNA
- Reliable pre-designed primers
- Wide selection of targets
- Suitable for amplification of bisulfite-modified DNA using the CpGenome™ Turbo Bisulfite kit

Detection of the methylation state of the p16 gene by a CpG WIZ® system



Detection of the Methylation State of the p16 Gene. MSP of the p16 gene in two invasive carcinomas, a squamous intraepithelial lesion (SIL), and an adenocarcinoma of the cervix. Both invasive carcinomas and the SIL sample are heterozygous for methylation while the adenocarcinoma sample is homozygous for the unmethylated state at the p16 locus.

Description	Catalogue No.
CpG WIZ® BRCA1 Amplification Kit	S7830
CpG WIZ® hMLH1 Amplification Kit	S7811
CpG WIZ® Fragile X Amplification Kit	S7807
CpG WIZ® MGMT Amplification Kit	S7803
CpG WIZ® GST-pi Amplification Kit	S7808
CpG WIZ® APC Amplification Kit	S7812
CpG WIZ® p14/ARF Amplification Kit	S7817
CpG WIZ® SOCS1 Amplification Kit	S7809
CpG WIZ® ERα Amplification Kit	S7815
CpG WIZ® DAP-Kinase Amplification Kit	S7801
CpG WIZ® Prader-Willi/Angelman Amplification Kit	S7806
CpG WIZ® RARβ2 Amplification Kit	S7814
CpG WIZ® p16 Amplification Kit	S7800
CpG WIZ® p15 Amplification Kit	S7802
CpG WIZ® E-Cadherin Amplification Kit	S7804
CpG WIZ® Oct-4 Amplification	S7840

For a complete list of CpG WIZ products, visit: **www.emdmillipore.com/epigenetics**

DNA Methylation Controls

Ensure accurate interpretation of DNA methylation data with reliable unmethylated, 5-mC, or 5-hmC DNA standards

CpGenome™ Human Methylated & Non-Methylated DNA Set

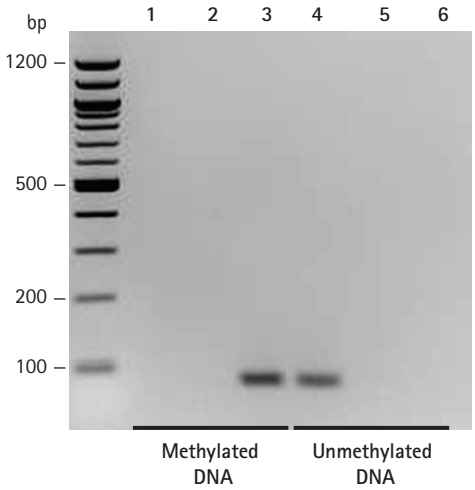
(Catalogue Nos. S8001, S8001U, and S8001M)

Primer specificity:

Lane 1,4:
Unmethylated, bisulfite mod.

Lane 2,5:
Wildtype, no bisulfite

Lane 3,6:
Methylated, bisulfite mod.



Human methylated and non-methylated DNA standards are amplified specifically by methylation-specific primers. DNA standards were bisulfite-modified using the CpGenome™ Turbo Bisulfite Kit (Catalogue No. S7847) and amplified using the CpG WIZ® BRCA1 Amplification Kit (Catalogue No. S7830).

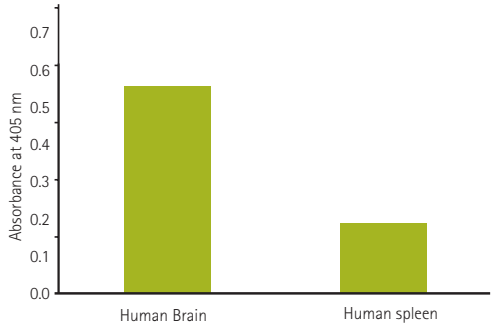
- Contains non-methylated human DNA purified from HCT116 cells with double-knockout alleles of DNA methyltransferases, DNMT1 (-/-) and DNMT3b (-/-)
- Methylated standard has all CpGs methylated by M.SssI methyltransferase
- Every lot sequenced and evaluated
- Fully compatible with CpGenome™ Turbo Bisulfite kit and CpG WIZ® systems
- **Available in three formats**:
  Non-methylated (Catalogue No. S8001U); Methylated (Catalogue No. S8001M); or a complete set (Catalogue No. S8001)

CpGenome™ 5-mC & 5-hmC Human DNA Standards

(Catalogue No. S8003)

Rapidly quantify levels of 5-hmC DNA in a wide range of DNA samples with a limit of detection down to 0.03% of input DNA.

The CpGenome™ 5-mC & 5-hmC Human DNA Standards provides two samples of human genomic DNA from the same individual. This genomic DNA contains physiological levels of 5-mC and 5-hmC,that have been precisely quantified by LC/MS analysis. These matched human standards are useful as positive controls in DNA methylation assays designed to detect or quantify 5-mC (5-methylcytosine) and/or 5-hmC (5-hydroxymethylcytosine) modifications.



Analysis of CpGenome™ 5-mC & 5-hmC Human DNA Standards

100 ng each of standard were evaluated using a hydroxymethylation quantitation assay (CpGenome™ 5-hmC Quantitation Kit 17-10091). The CpGenome 5-hmC Quantitation kit distinguished between different levels of 5-hmC in each standard previously determined by LC/MS analysis (human brain 1.89%; human spleen 0.18%).

CpGenome™ 5-mC and 5-hmC DNA Set

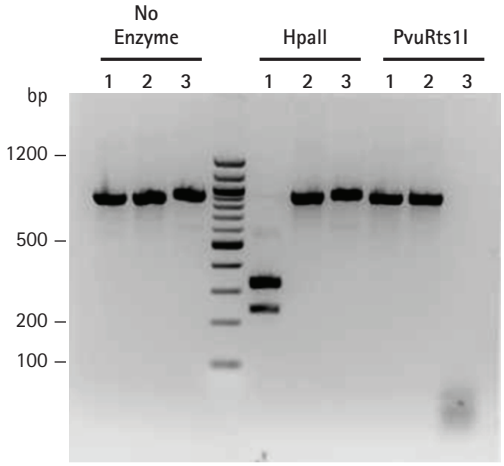
(Catalogue No. S8005, S8005H, S8005M, and S8005U)

- Linear double-stranded DNA (897 bp) of known sequence
- Each set includes three well-characterized standards:

  100% unmodified cytosines

  100% 5-hmC

  100% 5-mC
- Ideal for use as spike-in controls
- Easily calibrate applications to quantify modified cytosines
- Individual vials of each standard also available



Lane 1:
Unmethylated cytosine

Lane 2:
5-mC

Lane 3:
5-hmC

5-mC- and 5-hmC- modified DNA standards are cleaved by selective restriction enzymes. CpGenome™ 5hmC and 5mC standards were digested with HpaII (selectively cleaves unmethylated DNA) and PvuRts11, which selectively digests 5-hmC-modified DNA.

CpGenome™ Universal Methylated Mouse DNA

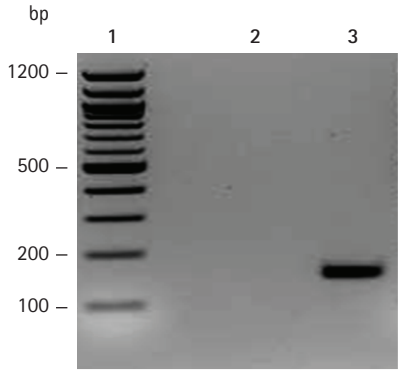
(Catalogue No. S8000)

Primer specificity:

Lane 1:
Unmethylated, bisulfite mod.

Lane 2:
Wildtype, no bisulfite

Lane 3:
Methylated, bisulfite mod.

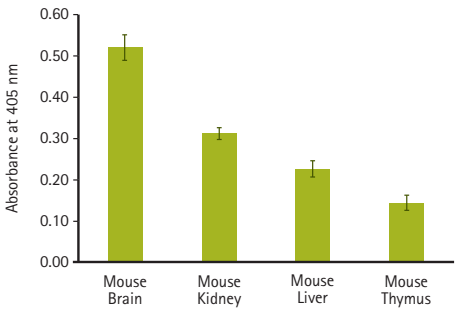


Mouse methylated DNA standard amplified only by methylation-specific primers. Universal Methylated Mouse DNA was modified using the CpGenome™ Turbo Bisulfite Kit (Catalogue No. S7847) and amplified by methylation-specific PCR with the EMD Millipore's CpG WIZ® Oct 4 Amplification Kit (Catalogue No. S7840).

- Mouse genomic DNA from male Balb/c mice enzymatically methylated by M.SssI methyltransferase
- Quality controlled by sequencing to ensure that all CpGs are methylated
- Fully compatible with CpGenome™ Turbo Bisulfite kit and CpG WIZ® systems

CpGenome™ 5-mC & 5-hmC Mouse DNA Standards

(Catalogue No. S8004)



100 ng each of mouse brain, kidney, liver, and thymus were evaluated with the CpGenome™ 5-hmC Quantitation Kit. Samples were developed for 30 minutes and read at 405 nm.

Rapidly quantify levels of 5-hmC DNA in a wide range of DNA samples with a limit of detection down to 0.03% of input DNA.

The CpGenome™ Mouse 5-mC and 5-hmC DNA Standards provides four samples of mouse genomic DNA from four different organs. This genomic DNA contains physiological levels of 5-mC and 5-hmC, which have been precisely quantified by LC/MS analysis. These standards are useful as positive controls in DNA methylation assays designed to detect or quantify 5-mC (5-methylcytosine) and/or 5-hmC (5-hydroxymethylcytosine) modifications.

Validated Antibodies
for MeDIP, hMeDIP and Other Applications

Description	Catalogue No.
5-Methylcytosine, 5-Hydroxymethylcytosine, and TET	
Anti-5-Hydroxymethylcytosine, clone AB3/63.3	MABE176
Anti-5-hydroxymethylcytosine (5hmC), clone HMC 31	MABE251
Anti-5-Methylcytosine, clone 33D3	MABE146
Anti-Methylcytosine Dioxygenase TET1	09-872
DNA Methyltransferases	
Anti-DNA Methyltransferase 3a (86-100)	
Rabbit pAb	317282-100UG
Anti-DNA Methyltransferase 1	AB3429
Anti-DNMT1	07-688
Anti-DNMT-1 Mouse mAb (60B1220.1)	ST1133-50UG
Anti-Phospho-DNMT1(Ser714)	07-1594
Anti-DNA Methyltransferase 3a	AB3431
Anti-DNMT3A2	07-2050
Anti-DNA Methyltransferase 3b	AB3433
Anti-CFP1	ABE211
Anti-DMAP1, C-terminus	07-2072
CpG-Binding Proteins	
Anti-MBD1 (methyl-CpG binding domain) protein 1	09-833
Anti-MBD1, C-terminus	07-2054
Anti-MBD2	07-198
Anti-MBD4	07-2057
Anti-acetyl-MeCP2 (Lys464)	ABE28
Anti-MeCP2 (Rabbit Polyclonal)	07-013
Anti-MeCP2 (Chicken Polyclonal)	ABE171
Selective Inhibitors	
DNA Methyltransferase Inhibitor	260920
DNA Methyltransferase Inhibitor II, SGI-1027	260921
Recombinant Protein	
Methylated DNA Binding Proteins and Modification Enzymes	
MBD4	14-1065
MECP2	14-1067
PvuRts1I - 5HMC endonuclease	14-1058
5-hmC Glucosyltransferase (200 units)	14-1047
CpG MethylQuest™ Protein (Recombinant GST-MBD fusion protein)	14-921

Related Products:
KOD DNA Polymerases for Efficient Amplification

KOD DNA polymerases meet the demands of epigenomic PCR analysis. KOD Hot Start DNA polymerase effectively amplifies CpG island regions following MSP[1].

KOD Xtreme™ Hot Start DNA polymerase efficiently amplifies promoter regions with up to 90% GC-rich DNA. This enzyme can be used to amplify gene targets from crude tissue lysates. Unlike many polymerases, KOD Xtreme™ polymerase is not limited to low alkaline pH following bisulfite treatment of DNA during methylationspecific PCR (MSP).

Reference:

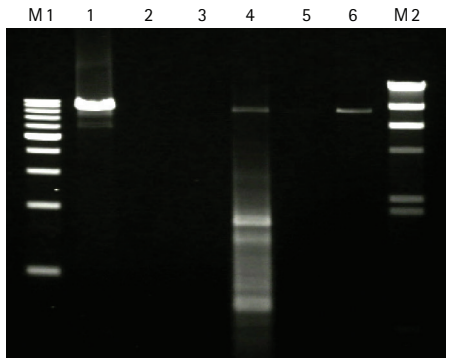
1. Hirai et al, Down-Regulation of Connexin 32 Gene Expression through DNA Methylation in Human Renal Cell Carcinoma. Am J Nephrol 2003; vol 23: 172-177.

Thermocycling Conditions

94 °C for 2 minutes	1 cycle
98 °C for 1 s 74 °C for 1min/kb	5 cycles
98 °C for 1 s 72 °C for 1 min/kb	5 cycles
98 °C for 10 s 70 °C for 1min/kb	5 cycles
98 °C for 10 s 68 °C for 1 min/kb	15 cycles

Table 1. Competitor polymerase PCR reactions were set up and thermocycling was performed according to manufacturers' protocols.

Amplify GC-Rich DNA with KOD Xtreme™ Hot Start DNA Polymerase



KOD Xtreme™ Hot Start DNA Polymerase amplifies GC-rich targets more efficiently than other polymerases. Six polymerases were used to amplify a 8.9 kb human IGF2R gene, containing ~90% GC content. Lane M1 and M2, markers; Lane 1, PCR using KOD Xtreme™ Hot Start DNA Polymerase (thermocycling as shown); Lanes 2 to 6, competitor polymerase systems supplied with GC Buffers and tested using manufacturer protocols. Data contributed by Akio Sugiyama, Tsuruga Institute of Biotechnology.

Description	Catalogue No.
KOD DNA Polymerase	71085-3
KOD Hot Start DNA Polymerase	71086
KOD Hot Start Master Mix	71842
KOD XL DNA Polymerase	71087-3
KOD Xtreme™ Hot Start DNA Polymerase	71975-3

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