EZ DNA Methylation-Gold™ Kit

Simple, Precise Bisulfite Conversion of DNA in 3 Hours!

Coupled Heat-Denaturation/ Bisulfite Treatment for Rapid Conversion of Non-Methylated Cytosine to Uracil

In-Column Desulphonation Technology

Input

500 pg – 2 µg DNA per treatment with 200 – 500 ng being optimal

Converted DNA is Ideal for Downstream Analysis Including…

- PCR
- Real-Time PCR
- Microarray
- Sequencing

Formats

- Single Column
- 96-well Format

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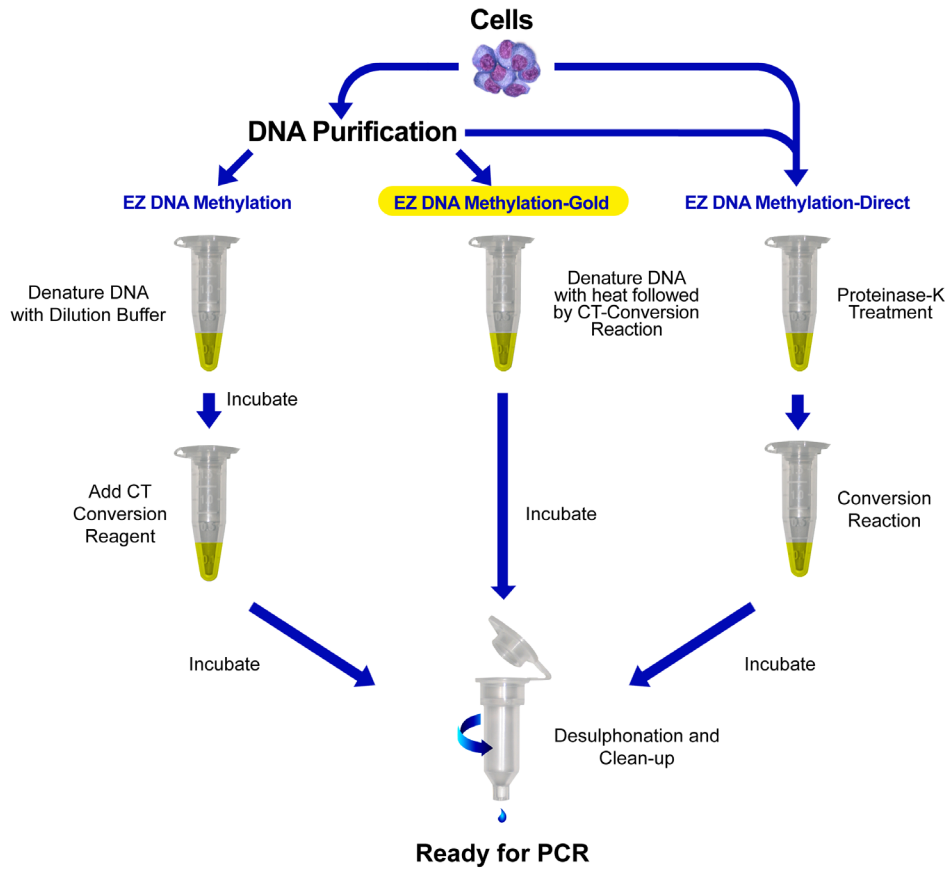
The **EZ DNA Methylation-Gold™ Kit** is a refinement of our popular **EZ DNA Methylation™ Kit**. The **EZ DNA Methylation-Gold™ Kit** integrates DNA denaturation and bisulfite conversion processes into a single step (see figure below). Also, the kit has been streamlined for high yield recovery of DNA following bisulfite treatment. Both kits are based on a three step reaction process between cytosine and sodium bisulfite resulting in cytosine being converted into uracil. The **EZ DNA Methylation-Gold™ Kit** features innovative in-column desulphonation technology that eliminates cumbersome DNA precipitation steps and provides consistent results every time. The kit has been designed to minimize template degradation, loss of DNA during treatment and clean-up, and to provide complete conversion of unmethylated cytosines. Recovered DNA is ideal for PCR, sequencing, microarrays, etc.

Outline of Zymo Research's current bisulfite conversion product



Comparative Overview

	EZ DNA Methylation-Gold™	Suppliers C & H
Processing Time	3 hrs.	16-20 hrs.
Protocol	Simple	Complicated
Conversion	Rapid, Complete - integrates denaturation/conversion into one step.	Slow, Incomplete - denaturation and conversion steps are separate.
Desulphonation	Convenient In-Column	Cumbersome Precipitation
DNA Recovery	High	Low
96-well Format	Yes	No

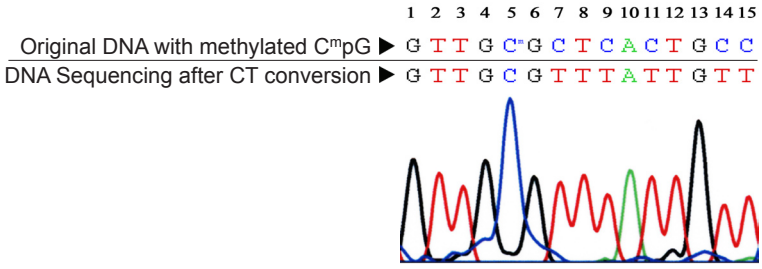
EZ DNA Methylation-Gold™ Kit

EZ DNA Methylation-Gold™ Kit Short Protocol

Preparation of CT Conversion Reagent:

Add 900 µl water, 50 µl of **M-Dissolving Buffer** and 300 µl of **M-Dilution Buffer** to one tube of **CT Conversion Reagent**. Mix for 10 minutes.

1. Add 130 µl of the prepared **CT Conversion Reagent** to 20 µl of DNA sample. Mix.
2. Perform the following temperature steps: 98°C for 10 minutes, 64° C for 2.5 hours, then hold at 4° C.
3. Add 600 µl of **M-Binding Buffer** to a **Zymo-Spin IC Column**, then add the sample. Close the cap and mix by inverting several times.
4. Centrifuge at full speed (≥10,000 x *g*) for 30 seconds. Discard the flow-through
5. Add 100 µl of **M-Wash Buffer** to the column, spin 30 seconds.
6. Add 200 µl of **M-Desulphonation Buffer** to the column and wait for 15-20 minutes.
7. Spin at full speed for 30 seconds.
8. Add 200 µl of **M-Wash Buffer** to the column, spin 30 seconds. Repeat this wash step one more time.
9. Add 10 µl of **M-Elution Buffer** directly to the column matrix. Place into a 1.5 ml tube. Spin briefly to elute the DNA



DNA sequencing results after bisulfite treatment. DNA with methylated C^{m}pG (at nucleotide position #5) was processed using the **EZ DNA Methylation-Gold™ Kit**. The recovered DNA was amplified by PCR and then sequenced directly. The methylated cytosine at position #5 remained intact while the unmethylated cytosines (i.e., positions #7, 9, 11, 14 and 15) were completely converted into uracil following bisulfite treatment and detected as thymine following PCR.

Selected Citations:

1. Takahiro Arima, *et al.* ZAC, LIT1 (KCNQ1OT1) and p57^{KIP2} (CDKN1C) are in an imprinted gene network that may play a role in Beckwith–Wiedemann syndrome. **Nucleic Acids Res.**, May 2005; 33: 2650 - 2660.
2. Hiroyuki Izumi, *et al.* Frequent silencing of DBC1 is by genetic or epigenetic mechanisms in non-small cell lung cancers. **Hum. Mol. Genet.**, Apr 2005; 14: 997 - 1007.
3. Siu-hong Chan, *et al.* Cloning of CviPII nicking and modification system fro chlorella virus NYs-1 and application of Nt.CviPII in random DNA amplification **Nucleic Acids Res.**, Nov 2004; 32: 6187 - 6199.
4. Remco van Doorn, *et al.* Epigenetic Profiling of Cutaneous T-Cell Lymphoma: Promoter Hypermethylation of Multiple Tumor Suppressor Genes Including BCL7a, PTPRG, and p73. **J. Clin. Oncol.**, May 2005; 10.1200 JCO.2005.11.353.
5. Michael T. McCabe, *et al.* Regulation of DNA Methyltransferase 1 by the pRb/E2F1 Pathway. **Cancer Res.**, May 2005; 65: 3624 - 3632.

Ordering Information

Kit	Catalog No.	Kit Size
EZ DNA Methylation-Gold™ Kit	D5005	50 Rxns.
EZ DNA Methylation-Gold™ Kit	D5006	200 Rxns.
EZ-96 DNA Methylation-Gold™ Kit	D5007	2x96 Rxns., 2x96 Shallow-well Silicon-A Plate™
EZ-96 DNA Methylation-Gold™ Kit	D5008	2x96 Rxns., 2x96 Deep-well Zymo-Spin I-96 Plate™

Methylated DNA Standards	Catalog No.	Kit Size
Universal Methylated DNA Standard	D5010	1 set
Universal Methylated Human DNA Standard	D5011	1 set
Universal Methylated Mouse DNA Standard	D5012	1 set

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