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ZYMO RESEARCH



5-Methylcytosine & 5-Hydroxymethylcytosine DNA Standard Set

Three identical DNA sequences with 100% of cytosines either unmodified, 5-mC, or 5-hmC.

Highlights

- Control DNA for 5-methylcytosine and 5-hydroxymethylcytosine quantitation applications (i.e., mass spectrometry, HPLC, TLC, etc.)
- Substrate for studies involving 5-mC and 5-hmC interacting proteins.

Catalog Numbers:

D5405



Scan with your smart-phone camera to view the online protocol/video.



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Product Contents

5-Methylcytosine & 5-Hydroxymethylcytosine DNA Standard Set	D5405	Storage Temp.
Cytosine DNA Standard	2 µg/40 µl	-20°C
5-Methylcytosine DNA Standard	2 µg/40 µl	-20°C
5-Hydroxymethylcytosine DNA Standard	2 µg/40 µl	-20°C

Specifications

- **Source** – Fully synthetic double-stranded DNA with cytosines either 100% unmodified, 100% methylated, or 100% hydroxymethylated at every C position.
- **Concentration** – 50 ng/µl in modified TE buffer (10mM Tris-HCl; 0.1mM EDTA; pH 8.0). All standards are supplied in 40 µl volume.

Product Description

The **5-Methylcytosine & 5-Hydroxymethylcytosine DNA Standard Set** is a set of three DNA standards that are linear, 897 bp dsDNA and have the same sequence (see **Figure 1** below and **Sequence Information** on pg. 4). The only difference is that each contains either 100% unmodified cytosines, 5-methylcytosines, or 5-hydroxymethylcytosines. Since the sequence and extent of cytosine modification is known, this DNA standard set is ideal for use in calibration of various applications intended for quantification of cytosine modifications. These DNA standards can also be used as substrates for studies involving 5-hydroxymethylcytosine interacting proteins¹.

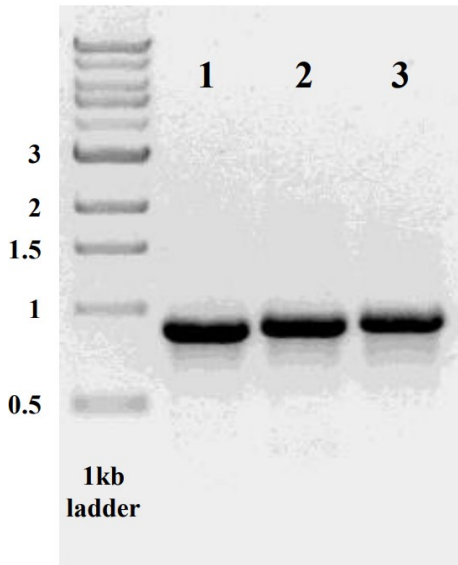


Figure 1. 5-Methylcytosine & 5-Hydroxymethylcytosine DNA Standard Set. All three standards are 897 bp with all cytosines either unmodified (lane 1, C), 5-methylcytosine (lane 2, 5-mC), or 5-hydroxymethylcytosine (lane 3, 5-hmC). Ladder used is **ZR 1kb DNA Marker (M5003)**.

¹Ito, S. *et al* (2010) "Role of Tet proteins in 5mC to 5hmC conversion, ES-cell self-renewal and inner cell mass specification". *Nature* 466, 1129-1133.

Recommended Usage

The **5-Methylcytosine & 5-Hydroxymethylcytosine DNA Standard Set** can be used as controls for bisulfite conversion and downstream analyses such as bisulfite PCR, methylation sequencing applications, HPLC, LC/MS, *in situ* controls, methylated DNA immunoprecipitation (MeDIP), and more.

Protocol

For best results, it's important to ensure the DNA is completely homogenous and fully in solution before quantification and usage. The following steps are recommended before quantification and usage¹:

1. Bring the standards to room temperature.
2. Vortex the standards for 10-15 seconds, briefly spin down for 5-10 seconds.
3. Repeat Step 2 twice, for three times total.
4. Proceed with quantification or usage.

¹Standards are quantified using NanoDrop® measurements. If using other methods, variation may be observed.

Appendix

Sequence Information

```

5'...1 AGAATTGGTT AATTGGTTGT AACACTGGCA GAGCATTACG CTGACTTGAC
  51 GGGACGGCGG CTTTGTGTA TAAATCGAAC TTTTGTGAG TTGAAGGATC
101 AGATCACGCA TCTTCCCGAC AACGCAGACC GTTCCGTGGC AAAGCAAAG
151 TTCAAAATCA CCAACTGGTC CACCTACAAC AAAGCTCTCA TCACCGTGG
201 CTCCCTCACT TTCTGGCTGG ATGATGGGGC GATTCAGGCC TGGTATGAGT
251 CAGCAACACC TTCTTACGGA GGCAGACCTC AGCGCTCAAA GATGCAGGGG
301 TAAAAGCTAA CCGCATCTTT ACCGACAAGG CATCCGGCAG TTCAACAGAT
351 CGGGAAGGGC TGGATTTGCT GAGGATGAAG GTGGAGGAAG GTGATGTCAT
401 TCTGGTGAAG AAGCTCGACC GTCTTGGCCG CGACACCGCC GACATGATCC
451 AACTGATAAA AGAGTTTGAT GCTCAGGGTG TAGCGGTTTCG GTTTATTGAC
501 GACGGGATCA GTACCGACGG TGATATGGGG CAAATGGTGG TCACCATCCT
551 GTCGGCTGTG GCACAGGCTG AACGCCGGAG GATCCTAGAG CGCACGAATG
601 AGGGCCGACA GGAAGCAAAG CTGAAAGGAA TCAAATTTGG CCGCAGGCGT
651 ACCGTGGACA GGAACGTCGT GCTGACGCTT CATCAGAAGG GCACTGGTGC
701 AACGGAAATT GCTCATCAGC TCAGTATTGC CCGCTCCACG GTTTATAAAA
751 TTCTTGAAGA CGAAAGGGCC TCGTGATACG CCTATTTTTA TAGGTTAATG
801 TCATGATAAT AATGGTTTCT TAGACGTCAG GTGGCACTTT TCGGGGAAAT
851 GTGCGCGGAA CCCCTATTTG TTTATTTTTTC TAAATACATT CAAATAT...3'

```

The sequence is the same for all three standards. Depending on the DNA, all C's are unmodified cytosines, 5-methylcytosine, or 5-hydroxymethylcytosine. The sequence can be downloaded on the product page of our website.

Sequence Statistics

Nucleotide	Count	Frequency (%)
Adenine (A)	237	26.4
Cytosine (C)	201	22.4
Guanine (G)	246	27.4
Thymine (T)	213	23.7
C + G	447	49.8
A + T	450	50.2

Ordering Information

Product Description	Catalog No.	Size
Human Methylated & Non-methylated DNA Set	D5014	5 µg/20 µl
Human HCT116 DKO Non-Methylated DNA	D5014-1	5 µg/20 µl
Human HCT116 DKO Methylated DNA	D5014-2	5 µg/20 µl
Bisulfite-Converted Universal Methylated Human DNA Standard	D5015	1 µg/50 µl
ZymoTaq™ qPCR Premix	E2054 E2055	50 Rxns. 200 Rxns.
ZymoTaq™ Premix	E2003 E2004	50 Rxns 200 Rxns.
EZ DNA Methylation-Lightning™ Kit	D5030 D5031	50 Rxns 200 Rxns.
EZ DNA Methylation-Direct™ Kit	D5020 D5021	50 Rxns 200 Rxns.
EZ DNA Methylation™ Kit	D5001 D5002	50 Rxns 200 Rxns.
EZ DNA Methylation-Gold™ Kit	D5005 D5006	50 Rxns 200 Rxns.



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