Universal methylated DNA standard (mouse) Protocol

Catalog number: ENZ-45006

Primer Set
mMLH1 Primer F:
5´ - GGTGTACGAAGTTATTTTTATTTTAGTC - 3´
mMLH1 Primer R:
5´ - ACCCAACGATACCTAATAATAAAACC - 3´

PCR Setup:
The following setup is designed for a 25 µl total reaction volume:

Component	Volume	Final Conc.
mMLH1 primer F*	Variable	0.2 to 0.8 µM
mMLH1 primer R*	Variable	0.2 to 0.8 µM
Bisulfite-converted DNA	2 µl	up to 20 ng/µl
10 mM dNTP mix	0.5 µl	0.2 mM each dNTP
Standard PCR buffer	Variable	1x
MgCl2 or MgSO4	Variable	1-4 mM, if needed
Hot-Start DNA Polymerase	1 to 2 units	
Hot-start DNA polymerase)	Variable 1 to 2 units	
Add water to 25 µl		

* Alternatively, you may substitute primers of your choice.
** Remember to bisulfite-treat the DNA prior to performing PCR.

Recommended Thermocycler Conditions:
95 °C, 10 minutes
95 °C, 30 seconds
58 °C, 30 to 60 seconds
72 °C, 60 seconds
Repeat steps B through D an additional 29 to 39 times
depending on the polymerase used.
72 °C, 7 minutes
4 °C
The PCR amplicon can now be used directly for sequencing analysis

Additional information including expected results

The expected PCR amplicon for the Universal Methylated Mouse DNA Standard is 304 bp, corresponding to nucleotide positions 430 to 778 of mouse MLH1 DNA including the regions (italicized) that hybridize to the primers (GenBank Accession #: AF400617).
Original sequence of mouse MLH1 DNA for bisulfite treatment and PCR amplification (sense strand 5' to 3'). The cytosines (underlined) in the CpG dinucleotide context (bold capital letters) are methylated enzymatically by M.SssI methyltransferase:

421 ---------*g gtgta**CG**aag tcaccctcac cccagc**CGCG***
461 acccttcaag gccaagaag**C G**gcagaggc**C G**aggcctgcc
501 **CGCG**t**CG**ctc tctcctc**CG**g agtgagca**CG** g**CG**gccaaag
541 acatgtcacc ctgc**CG**caga **CG**ct**CG**acca gggc**CGCGCG**
581 ttcct**CG**tcc cctacaaac**C G**ct**CG**tagaa tt**CG**tgct**CG**
621 gcct**CG**tagt gg**CG**cctca**C G**t**CGCG**ttcc **CG**agtagagg
661 **CG**accagg**CG** g**CG**acacacc aggcacaggg ccc**CG**tcacc
701 ctc**CG**c*aggc tccaccacca ggtat**CG**ctg ggt*-------

Expected sequence of the above DNA following bisulfite treatment. Methylated cytosines in the CpG dinucleotide context remain unconverted following bisulfite treatment, whereas non-methylated cytosines, or cytosines not in the CpG context, are converted to uracil and detected as thymine after PCR.

421 ---------*g gtgta**CG**aag ttatttttat tttagt**CGCG***
461 Atttttaag gttaagaag**C G**gtagaggt**C G**aggtttgtt
501 **CGCG**t**CG**ttt ttttttt**CG**g agtgagta**CG** g**CG**gttaaag
541 atatgttatt ttgt**CG**taga **CG**tt**CG**atta gggt**CGCGCG**
581 ttttt**CG**ttt tttataaat**C G**tt**CG**tagaa tt**CG**tgtt**CG**
621 gttt**CG**tagt gg**CG**ttttta**C G**t**CGCG**tttt **CG**agtagagg
661 **CG**attagg**CG** g**CG**atatatt aggtataggg ttt**CG**ttatt
701 ttt**CG**ta*ggt tttattatta ggtat**CG**ttg ggt*-------