Universal methylated DNA standard (human) Protocol

Catalog number: ENZ-45005

Primer Set
hMLH1 Primer F:
5´ - GGAGTGAAGGAGGTTACGGGTAAGT - 3´
hMLH1 Primer R:
5´ - AAAAACGATAAAACCCTATACCTAATCTATC - 3´

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

Component	Volume	Final Conc.
hMLH1 primer F*	Variable	0.2 to 0.8 µM
hMLH1 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 Human DNA Standard is 182 bp, corresponding to nucleotide positions 804 to 986 of human MLH1 DNA including the regions (italicized) that hybridize to the primers (GenBank Accession #: U83845).
Original sequence of human MLH1 DNA for bisulfite treatment and PCR amplification (sense strand 5' to 3'). The cytosines (underlined) in the CpG dinucleotide context (bold capitol letters) are methylated enzymatically by M.SssI methyltransferase:
801 ---*ggagtga aggaggcca**C** **G**gcaagt***CG*** ccctga***CG***ca
841 ga***CG***ctccac cagggc***CGCG*** ***CG***ct***CG***c***CG***t c***CG***ccacata
881 c***CG***ct***CG***tag tatt***CG***tgct cagcct***CG***ta gtgg***CG***cctg
921 a***CG***t***CGCG***tt ***CGCG***ggtagc ta***CG***atgagg ***CG***g***C*****G***acaga*
*961 ccaggcacag ggccccat**CG** ccctc*----- ----------

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

801 ---*ggagtga aggaggtta**C** **G**gtaagt***CG*** ttttga***CG***ta
841 ga***CG***ttttat tagggt***CGCG*** ***CG***tt***CG***t***CG***t t***CG***ttatata
881 t***CG***tt***CG***tag tatt***CG***tgtt tagttt***CG***ta gtgg***CG***tttg
921 a***CG***t***CGCG***tt ***CGCG***ggtagt ta***CG***atgagg ***CG***g***C*****G***ataga*
*961 ttaggtatag ggttttat**CG** ttttt*-----