OPTI-Bisulfite treatment protocol

For up to 10 reactions:

- weigh 58 mg bisulfite powder and solve completely in 763.5 µl fresh MilliQ
- add 183.5 µl 3M NaOH to the bisulfite solution
- weigh 14 mg scavenger powder and solve completely in 394.8 µl 1,4-Dioxane

For up to 20 reactions:

- weigh 1.16 g bisulfite powder and solve completely in 1.527 ml fresh MilliQ
- add 367 µl 3M NaOH to the bisulfite solution
- weigh 28 mg scavenger powder and solve completely in 789.6 µl 1,4-Dioxane

Sulfonation and deamination:

- add 94 µl properly mixed bisulfite/NaOH solution and 37 µl scavenger solution to 10 µl DNA (100-300ng)
- perform the following cycler program in 0.2 ml tubes in a thermal cycler (make sure that the complete reaction volume is inside the thermal block):
- 15 min 99°C
- 30 min 50°C
- 5 min 99°C } 2 cycles
- 90 min 50°C }

Washing, Desulfonation and Elution:

- pipet 400 µl M-Binding Buffer on a Zymo-Spin™ IC column and add complete sulfonation reaction
- centrifuge 30s at 13.000 rpm, discard flow-through
- pipet 100 µl M-Wash Buffer to the column and centrifuge 30s at 13.000 rpm
- pipet 200 µl M-Desulfonation Buffer to the column and incubate 20 min
- centrifuge 30s at 13.000 rpm
- pipet 200 µl M-Wash Buffer to the column and centrifuge 30s at 13.000 rpm
- pipet 200 µl M-Wash Buffer to the column and centrifuge 30s at 13.000 rpm
- place column in a fresh 1.5ml tube and pipet 20 µl M-Elution Buffer or MilliQ onto the membrane
- to elute bisulfite-converted DNA, centrifuge 30s at 13.000 rpm

In case DNA amount is low or when sequencing library preparation is performed (e.g. RRBS) elution volume can be reduced to 10 µl !

Materials:

Sodium metabisulfite	VWR	Darmstadt, Germany
Scavenger (Trolox)	Sigma-Aldrich	St.Louis, Missouri, USA
NaOH	Sigma-Aldrich	St.Louis, Missouri, USA
1,4-Dioxane	VWR	Darmstadt, Germany
Zymo-Spin™ IC columns	Zymo Research	Irvine, California, USA
M-Binding Buffer	Zymo Research	Irvine, California, USA
M-Wash Buffer	Zymo Research	Irvine, California, USA
M-Desulfonation Buffer	Zymo Research	Irvine, California, USA
M-Elution Buffer	Zymo Research	Irvine, California, USA