PvuRts1 I restriction enzyme

Catalog No: 55011

Quantity: 50 Unit(s)
Concentration: 1,000 Unit(s)/mL

Reagents Supplied: PvuRts1 I restriction enzyme
10X PvuRts1 I Reaction Buffer
1 M DTT

Background: In mammalian genomes, DNA methylation usually occurs at the fifth carbon of cytosine residues. The recent discovery that the TET family of iron-dependent deoxygenases can convert 5-methylcytosine (5-mC) into 5-hydroxymethylcytosine (5-hmC) has raised questions about the functional relevance of 5-hmC in mammalian genomes. The **PvuRts1 I (PvuRts1I) restriction enzyme** serves as a valuable tool to analyze 5-hmC patterns within the genome. PvuRts1 I is able not only to distinguish between 5-mC and 5-hmC DNA, but it can also cleave both glucyosylated and non-glucosylated 5-hmC DNA.

Enzyme Recognition Site: $^{hm}CN_{11\text{-}12}/N_{9\text{-}10}G$

Source: *E. coli* containing plasmid encoding the PvuRts1 I (PvuRts1I) sequence.

Unit Definition: One unit is defined as the amount of enzyme required to fully cleave 1 µg of 5-hmC DNA standard (Catalog No. 55008) in 30 minutes at 22°C in a total reaction volume of 30 µl. A pilot titration of enzyme is recommended for cleavage of genomic DNA.

Reaction Conditions: DTT should be used at a 1 mM final concentration for each reaction. Prepare a fresh dilution of the provided 1 M DTT to 10 mM working stock in sterile water before use
(e.g. add 10 µl 1M DTT to 990 µl sterile water to make 1 ml of a 10 mM DTT working solution). Discard any unused 10 mM DTT solution after use.

Example reaction:	
Sterile water	varies
10X Reaction Buffer	3 µl
10 mM DTT solution	3 µl
Sample DNA	varies
PvuRts1 I enzyme	1 µl
Total Volume	30 µl

Incubate at 22°C for 30 minutes.

Heat Inactivation: 65°C for 10 minutes

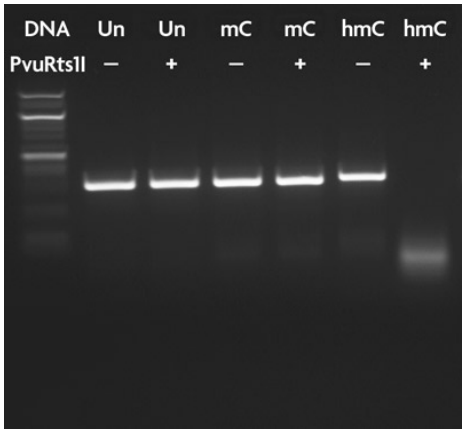

Figure 1: One µg of unmethylated (Un), 5-methylcytosine (mC) or 5-hydroxymethylcytosine (hmC) Methylated DNA Standard (Catalog No. 55008) was incubated in the absence or presence of 1 unit PvuRts1 I enzyme for 30 minutes at 22°C. Each reaction was run on a 2.5% agarose gel alongside a 1 kb DNA ladder.

Applications: PvuRts1 I can be used to directly cleave hydroxymethylated DNA (5-hmC) in its non-glucosylated form. The PvuRts1 I enzyme also digests α- and β-glucyosylated-hmC DNA, but at a lower efficiency than the non-glucosylated form. The enzyme is specific to 5-hmC DNA and will not digest 5-methylcytosine residues or unmethylated DNA (Figure 1). The enzyme is best suited for use in regions of high 5-hmC density, such as gene bodies.

General Notes: The PvuRts1 I restriction enzyme makes one cut in the recognition sequence generating a 2 nucleotide 3' overhang cleavage pattern. The enzyme does not produce defined DNA ends upon cleavage.

Reference: Szwagierczak, A. *et al.*, (2011) doi:10.1093/nar/gkr118.

Storage and Guarantee: The PvuRts1 I enzyme is supplied in 50% glycerol and can be stored at -20°C. Make small aliquots and store at -80°C for prolonged storage. This product is guaranteed stable for 6 months from date of receipt when stored properly.