TECHNICAL DATA SHEET

PRODUCT NAME PvuRts1I restriction enzyme		
Cat. No. : AF-113-0100	**Format :** 100 U/ 100 µl	**Concentration :** 1 U/µl

Product description:
5-Hydroxymethylcytosine (5-hmC) (called the sixth base) was recently described as a stable modification in mammalian DNA. This new cytosine modification results from the enzymatic conversion of 5-methylcytosine (5-mC) into 5-hydroxymethylcytosine by the TET family of oxygenases. Overwhelming evidence supports the idea that 5-hydroxymethylcytosine may represent a new pathway to demethylate DNA involving complex active mechanisms converting 5-hmC to cytosine.

Obtaining a specific assay for 5-hmC is particularly important since standard methods (eg. bisulfite sequencing) cannot distinguish between these two types of methylation. Used in conjunction with β-glucosyltransferase (Cat No. AF-112-0100) restriction enzymes such as PvuRts1I serve as valuable tools to discriminate between 5-mC and 5-hmC bases.

PvuRts1I is a restriction enzyme that selectively cleaves 5-hmC-containing DNA sequences [1]. PvuRts1I is able not only to discriminate between 5-hmC and 5-mC but it can also cleave both glucosylated and non-glucosylated 5-hmC DNA templates [2].

The consensus cleavage site of PvuRts1I is hmCN11–12/N9–10G. A recent report showed first data on its potential to interrogate 5-hmC patterns in mammalian genomes [2].

Features:
- Isolated from a recombinant source
- selectively cleaves 5-hmC-containing DNA sequences
- cleave both glucosylated and non-glucosylated 5-hmC DNA sequences
- Supplied with optimized 10X Reaction Buffer
- Can be used to assess the relative abundance of 5-hmC in genomic DNA [2]
- Ideal to control hMeDIP experiments

Source: Purified from an E. coli strain, which carries a PvuRts1I gene construct.

Heat inactivation: 65°C for 10-15 minutes

Shipping conditions: Shipped on dry ice.

Storage conditions: Storage at -80°C is highly recommended. Since the 10x reaction buffer contains DTT, it is also highly recommended to store it in small aliquotes at -80°C. Multiple freeze thawing of both the enzyme and its 10x reaction buffer should in any case be avoided. When stored properly the enzyme is stable for at least 6 months from date of receipt.

Package content:
- 1 tube of 100µl of PvuRts1I enzyme
- 1 tube of 300µl of 10 X PvuRts1I reaction buffer (enough for 120 x 25µl assays)

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Unit definition:
1 unit is the amount of enzyme required to cleave 1µg of fully 5-hmC DNA in 20minutes at 25ºC in 1x PvuRts1I reaction buffer in a total volume of 50µl.

Reaction Conditions:
Thaw reagents completely and put them on ice. Mix all reagents well before use.

For a 25 µl glucosylation reaction :

10x PvuRts1I reaction buffer:	2.5 µl
DNA template:	1 µg
PvuRts1I restriction enzyme(1 U/µl) :	1 µl
H_2O:	up to 25 µl

Incubate the reaction at 32°C for 30 minutes. If needed the incubation time can be further extended to 1 hour.

Application:
- PvuRts1I cleaves hydroxymethylated DNA (5-hmC). The enzyme is specific to 5-hmc DNA and will not digest 5-methylcytosine or unmethylated DNA (Figure 1).
- PvuRts1I can also digests glucosylated-hmC DNA (Figure 2).
- Ideal to control hMeDIP experiments (Figure 3).

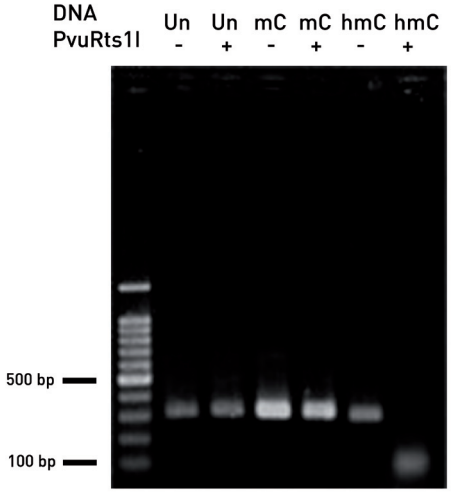


Figure 1
500 ng of hydroxymethylated, methylated and unmethylated DNA standards (Cat. No. AF-101-002) were incubated in the absence or presence of 1 unit PvuRts1I enzyme for 30 minutes at 32°C. Each sample was run on a 1.5% agarose gel with a 100 bp DNA ladder.

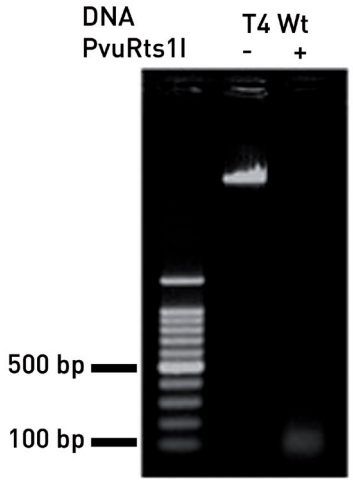
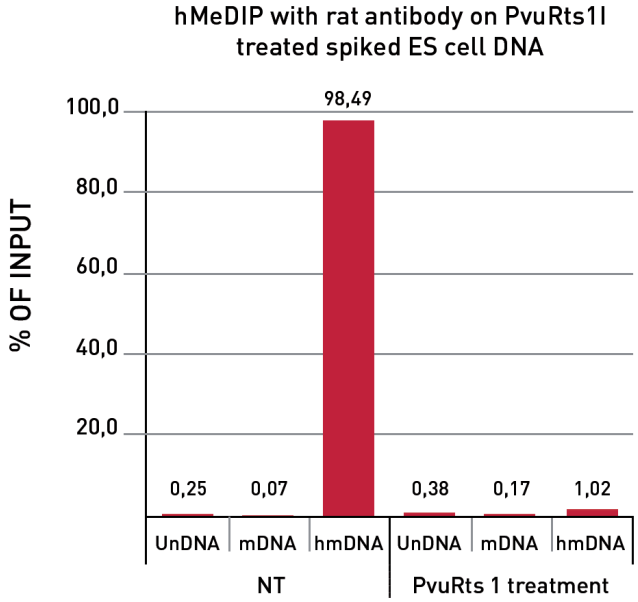


Figure 2

One µg of T4wt (glucosylated) genomic DNA was incubated in the absence or presence of 1 unit PvuRts1I enzyme overnight at 32°C. Each sample was run on a 1.5% agarose gel with a 100 bp DNA ladder.

Figure 3



Mouse embryonic stem cell DNA was fragmented by sonication using the Diagenode Bioruptor® (size range: 100-500 bp). One µg of sheared mouse embryonic stem cells was mixed with the three DNA controls (C, 5-mC and 5-hmC) from the hMeDIP kit and incubated with (PvuRts 1 treatment) or without (NT) 1 unit PvuRts1I restriction enzyme for 30 minutes at 32 °C. After purification with the PrepEaseTM PCR Purification 96-well Plate kit (PN 78761, USB®), this reaction was recovered in 108 µl nuclease-free water and added to 12 µl buffer H1 from the hMeDIP kit (Cat. No. AF-104-0016). The IP incubation mix was then heat denatured and hydroxymethylated DNA IP (hMeDIP) assays were performed using the Diagenode hMeDIP kit (Cat. No. AF-104-0016). This kit includes: the monoclonal rat antibody & Rat IgG (Cat. No. AF-105-0025). Finally qPCR using specific primer pairs for the unmethylated, methylated and hydroxymethylated DNA sequences were performed. After PvuRts1I enzymatic treatment, the recovery of the 5-hmC control is almost completely abolished.

Related products:

• β-glucosyltransferase	Cat. No AF-112-0100
• 5-hmC, 5-mC & cytosine DNA standard pack (2µg)	Cat. No AF-101-0002
• hMeDIP kit x16 (for monoclonal mouse antibody)	Cat. No AF-110-0016
• hMeDIP kit x16 (for monoclonal rat antibody)	Cat. No AF-104-0016
• hMeDIP kit x16 (for polyclonal rabbit antibody)	Cat. No AF-111-0016
• 5-hmC monoclonal antibody (rat)	Cat. No : MAb-633HMC-100
• 5-hmC monoclonal antibody (mouse)	Cat. No : MAb-31HMC-100
• 5-hmC polyclonal antibody – crude (rabbit)	Cat. No : CS-HMC-100
• 5-hmC polyclonal antibody – purified (rabbit)	Cat. No : pAB-HMC-100

Important remark:

The amount of enzyme required to obtain complete digestion of 5-hmC-containing fragments is dependant upon the degree of hydroxymethylation in your sample DNA. Therefore some optimization might be required depending on the type or size of DNA used.

References:

1. Janosi L., Yonemitsu H., Hong H., Kaji A. Molecular cloning and expression of a novel hydroxymethylcytosine-specific restriction enzyme (PvuRts1I) modulated by glucosylation of DNA. J Mol Biol. 1994 Sep 9;242(1):45-61.
2. Szwagierczak A, Brachmann A, Schmidt CS, Bultmann S, Leonhardt H, Spada F. Characterization of PvuRts1I endonuclease as a tool to investigate genomic 5-hydroxymethylcytosine. Nucleic Acids Res. 2011 Mar 4. [Epub ahead of print]

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