Restriction Endonuclease

Kro I

E541

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50 u **Lot: 2**
1000 u/ml **Store at -20°C**

Recognition sequence:

5'-G↓C(5mC) GGC-3'
3'-CGG(5mC)C↑G-5'

Sourse: ***Kocurea rosea*** **307**

Warranty period for the enzyme storage at-20˚C is one year from the date of the last assay indicated on the enzyme vial.

Supplied in:
10 mM Tris-HCl (pH 7.5); 200 mM NaCl; 0,1 mM EDTA; 200 μg/ml BSA; 7 mM 2-mercaptoethanol; 50% glycerol.

Reaction Conditions:
1×SEBuffer **G**. Incubate at **37°C**.

1×SEBuffer G (pH 7.6 @ 25ºC)
10 mM Tris-HCl 50 mM NaCl
10 mM $MgCl_2$ 1 mM DTT

Substrate specificity:
The enzyme cleaves C5-methylated DNA and doesn't cut unmodified DNA [1]. Kro I doesn`t cleave DNA modified with MspI DNA-methyltransferase.

Unit Definition: One unit is defined as the amount of enzyme required to hydrolyze completely1 μg of linearized plasmid pMHpaII 1 in 1 hour at 37°C in a total reaction volume of 50 μl.

DNA pMHpaII 1/DriI is a linearized plasmid pMHpaII 1. pMHpaII 1 carries a gene of DNA-methyltransferase M.HpaII, which methylates sites 5`-CCGG-3` producing 5`-C(5mC)GG-3`/3`-GG(5mC)C-5`, and includes three canonical sites 5`-GC(5mC)GGC-3`/3`-CGG(5mC)CG-5`.

Reagents Supplied with Enzyme: 10×SEBuffer G
Heat Inactivation: Yes (65°C for 20 minutes)

Quality Control Assays

16-Hour Incubation:
No detectable degradation of 1μg of Lambda DNA was observed after incubation with 1 units of enzyme for 16 hours at 37°C in a total reaction volume of 50 μl.

Oligonucleotide Assay:
No detectable degradation of a single- and double-stranded oligonucleotide was observed after incubation with 1 units of enzyme for 3 hours.

Enzyme Properties

Activity in SEBuffers:

SEBuffer B 50-75%
SEBuffer G 100%
SEBuffer O 25-50%
SEBuffer W 50-75%
SEBuffer Y 75-100%
SEBuffer ROSE 100%

When using a buffer other than the optimal (suppied) SEBuffer, it may be necessary to add more enzyme to achieve a complete digestion.

References:

1. ***Chernukhin V.A., Zhuravleva R.O., Tarasova G.V., Boltengagen A. A., Akishev A.G., Mikhnenkova N.A., Degtyarev S.Kh.*** **Bacterial strain Kocuria rosea - producer of KroI site specific endonuclease.** // Russian Federation patent RU 2394099 C1 (2010).

CERTIFICATE OF ANALYSIS