HpyCH4III

NEW ENGLAND BioLabs Inc.
1-800-632-7799
info@neb.com
www.neb.com

R0618S 001120814081

R0618S

250 units **5,000 U/ml** **Lot: 0011208**

RECOMBINANT Store at –20°C Exp: 8/14

Recognition Site:

5′... A C N▼G T ...3′
3′... T G▲N C A ...5′

Source: An *E. coli* strain that carries the cloned HpyCH4III gene from *Helicobacter pylori* CH4 (S. A. Thompson)

Supplied in: 100 mM NaCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 100 µg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme:
10X NEBuffer 4.

Reaction Conditions: 1X NEBuffer 4. Incubate at 37°C.

1X NEBuffer 4:
50 mM potassium acetate
20 mM Tris-acetate
10 mM magnesium acetate
1 mM dithiothreitol
pH 7.9 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme required to digest 1 µg of λ DNA in 1 hour at 37°C in a total reaction volume of 50 µl.

Diluent Compatibility: Diluent Buffer A
50 mM KCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C).

Quality Control Assays

Ligation: After 5-fold overdigestion with HpyCH4III, approximately 50% of the DNA fragments can be ligated with T4 DNA Ligase (at a 5′ termini concentration of 1–2 µM) at 16°C. Of these ligated fragments, > 95% can be recut.

16-Hour Incubation: A 50 µl reaction containing 1 µg of DNA and 50 units of enzyme incubated for 16 hours resulted in the same pattern of DNA bands as a reaction incubated for 1 hour with 1 unit of enzyme.

Exonuclease Activity: Incubation of 15 units of enzyme with 1 µg sonicated [^{3}H] DNA (10^5 cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer released < 0.1% radioactivity.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	100%
NEBuffer 2	50%
NEBuffer 3	25%
NEBuffer 4	**100%**

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

Survival in a Reaction: A minimum of 0.13 unit is required to digest 1 µg of substrate DNA in 16 hours.

Heat Inactivation: 10 units of enzyme were inactivated by incubation at 80°C for 20 minutes.

Notes: Bst4CI is an isoschizomer of HpyCH4III.

HpyCH4III produces DNA fragments that have a single-base 3′ extension which are difficult to ligate. More efficient ligation can be achieved by using the Quick Ligation Kit (NEB #M2200).

Not sensitive to *dam*, *dcm* or mammalian CpG methylation

Overdigestions > 4 hours with > 5.0 units per µg DNA are not recommended.

U.S. Patent No. 6,238,904

CERTIFICATE OF ANALYSIS

HpyCH4III

NEW ENGLAND BioLabs Inc.
1-800-632-7799
info@neb.com
www.neb.com

R0618S 001120814081

R0618S

250 units 5,000 U/ml Lot: 0011208

RECOMBINANT Store at –20°C Exp: 8/14

Recognition Site:

5′... A C N▼G T ...3′
3′... T G▲N C A ...5′

Source: An *E. coli* strain that carries the cloned HpyCH4III gene from *Helicobacter pylori* CH4 (S. A. Thompson)

Supplied in: 100 mM NaCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 100 µg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme:
10X NEBuffer 4.

Reaction Conditions: 1X NEBuffer 4. Incubate at 37°C.

1X NEBuffer 4:
50 mM potassium acetate
20 mM Tris-acetate
10 mM magnesium acetate
1 mM dithiothreitol
pH 7.9 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme required to digest 1 µg of λ DNA in 1 hour at 37°C in a total reaction volume of 50 µl.

Diluent Compatibility: Diluent Buffer A
50 mM KCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C).

Quality Control Assays

Ligation: After 5-fold overdigestion with HpyCH4III, approximately 50% of the DNA fragments can be ligated with T4 DNA Ligase (at a 5′ termini concentration of 1–2 µM) at 16°C. Of these ligated fragments, > 95% can be recut.

16-Hour Incubation: A 50 µl reaction containing 1 µg of DNA and 50 units of enzyme incubated for 16 hours resulted in the same pattern of DNA bands as a reaction incubated for 1 hour with 1 unit of enzyme.

Exonuclease Activity: Incubation of 15 units of enzyme with 1 µg sonicated [^{3}H] DNA (10^5 cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer released < 0.1% radioactivity.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	100%
NEBuffer 2	50%
NEBuffer 3	25%
NEBuffer 4	**100%**

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

Survival in a Reaction: A minimum of 0.13 unit is required to digest 1 µg of substrate DNA in 16 hours.

Heat Inactivation: 10 units of enzyme were inactivated by incubation at 80°C for 20 minutes.

Notes: Bst4CI is an isoschizomer of HpyCH4III.

HpyCH4III produces DNA fragments that have a single-base 3′ extension which are difficult to ligate. More efficient ligation can be achieved by using the Quick Ligation Kit (NEB #M2200).

Not sensitive to *dam*, *dcm* or mammalian CpG methylation

Overdigestions > 4 hours with > 5.0 units per µg DNA are not recommended.

U.S. Patent No. 6,238,904

CERTIFICATE OF ANALYSIS