CspCI

1-800-632-7799
info@neb.com
www.neb.com

R0645S 0041206130 61

R0645S

NEB4 37° SAM Yes

500 units **5,000 U/ml** **Lot: 0041206**

RECOMBINANT **Store at –20°C Exp: 6/13**

Recognition Site:

5′. . .▼$_{11}$(N) C A A (N)$_5$ G T G G (N)$_{12}$▼. . . 3′
3′. . .$_{13}$▲(N) G T T (N)$_5$ C A C C (N)$_{10}$▲. . . 5′

or

5′. . .▼$_{10}$(N) C A A (N)$_5$ G T G G (N)$_{12}$▼. . . 3′
3′. . .$_{12}$▲(N) G T T (N)$_5$ C A C C (N)$_{10}$▲. . . 5′

or

New Reaction Buffer

5′. . .▼$_{11}$(N) C A A (N)$_5$ G T G G (N)$_{13}$▼. . . 3′
3′. . .$_{13}$▲(N) G T T (N)$_5$ C A C C (N)$_{11}$▲. . . 5′

or

5′. . .▼$_{10}$(N) C A A (N)$_5$ G T G G (N)$_{13}$▼. . . 3′
3′. . .$_{12}$▲(N) G T T (N)$_5$ C A C C (N)$_{11}$▲. . . 5′

Source: An *E. coli* strain that carries the cloned CspCI gene from *Citrobacter* species 2144 (C. Nkenfou)

Supplied in: 100 mM NaCl, 10 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme:
10X NEBuffer 4, 1600X S-adenosylmethionine (SAM) (32 mM).

Reaction Conditions: 1X NEBuffer 4, supplemented with 20 µM S-adenosylmethionine (SAM supplied). 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

16-Hour Incubation: A 50 µl reaction containing 1 µg of λ DNA and 10 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 50 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	10%
NEBuffer 2	**100%**
NEBuffer 3	10%
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 0.5 unit is required to digest 1 µg of substrate DNA in 16 hours.

Heat Inactivation: 65°C for 20 minutes.

Notes: S-adenosylmethionine or SAM is supplied as a 32 mM solution in 0.005 M sulfuric acid and 10% ethanol. Under these conditions SAM is stable for up to 6 months when stored at –20°C.

CspCI cleaves DNA substrates twice to excise its recognition site generating a 35 base-pair fragment with 2-base 3′ overhangs.

(see other side)

CERTIFICATE OF ANALYSIS

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R0645S 0041206130 61
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NEB4 37° SAM Yes
500 units 5,000 U/ml Lot: 0041206
RECOMBINANT Store at –20°C Exp: 6/13
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3′. . .$_{12}$▲(N) G T T (N)$_5$ C A C C (N)$_{10}$▲. . . 5′
or
New Reaction Buffer
5′. . .▼$_{11}$(N) C A A (N)$_5$ G T G G (N)$_{13}$▼. . . 3′
3′. . .$_{13}$▲(N) G T T (N)$_5$ C A C C (N)$_{11}$▲. . . 5′
or
5′. . .▼$_{10}$(N) C A A (N)$_5$ G T G G (N)$_{13}$▼. . . 3′
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Reagents Supplied with Enzyme: 10X NEBuffer 4, 1600X S-adenosylmethionine (SAM) (32 mM).
Reaction Conditions: 1X NEBuffer 4, supplemented with 20 µM S-adenosylmethionine (SAM supplied). 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.
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16-Hour Incubation: A 50 µl reaction containing 1 µg of λ DNA and 10 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.
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NEBuffer 1 10%
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CspCI cleaves DNA substrates twice to excise its recognition site generating a 35 base-pair fragment with 2-base 3′ overhangs.
(see other side)
CERTIFICATE OF ANALYSIS

The cleavage point may shift one base pair depending on the DNA sequence context before and after the recognition site. For a given sequence, one site will predominate. For details, see www.neb.com.

Requires S-adenosylmethionine for optimal activity (supplied with enzyme).

= Time-Saver™ Qualified (See www.neb.com for details).

U.S. Patent No. 7,247,464

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