BtgZI

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R0703S 005121214121

R0703S

100 units **Lot: 0051212** **Exp: 12/14**
5,000 U/ml **Store at –20°C**

Recognition Site:

5′... G C G A T G $(N)_{10}$▼... 3′
3′... C G C T A C $(N)_{14}$▲... 5′

Source: An *E. coli* strain that carries the cloned BtgZI gene from *Bacillus thermoglucosidasius* (X. Pan)

2X More Units

Supplied in: 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme:
10X NEBuffer 4, 100X BSA.

Reaction Conditions: 1X NEBuffer 4, supplemented with 100 µg/ml BSA.
Incubate at 60°C.

1X NEBuffer 4:
50 mM potassium acetate
20 mM Tris acetate
10 mM magnesium acetate
1 mM DTT
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 60°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 BtgZI, approximately 75% 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, > 75% can be recut.

16-Hour Incubation: A 50 µl reaction containing 1 µg of λ DNA and 8 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 2 units of enzyme with 1 µg sonicated ^{3}H DNA (2 x 10^5 cpm/µg) for 4 hours at 60°C in 50 µl reaction buffer released < 0.2% radioactivity.

Endonuclease Activity: Incubation of 6 units with 1 µg pUC19 DNA for 4 hours at 60°C in 50 µl reaction buffer resulted in < 10% conversion to RF II.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	10%
NEBuffer 2	25%
NEBuffer 3	0%
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 1.0 unit is required to digest 1 µg of substrate DNA in 16 hours.

Heat Inactivation: 80°C for 20 minutes.

Notes: Cleavage of mammalian genomic DNA is blocked by CpG methylation.

Incubation at 37°C results in 75% activity.

BtgZI can remain bound to DNA after cutting and alter migration rate of DNA during electrophoresis. To disrupt binding, add SDS to a final concentration of 0.5% or purify DNA before electrophoresis.

U.S. Patent No. 7,029,900

CERTIFICATE OF ANALYSIS

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