BglII

Catalogue number:

MB06501, 1000 U
MB06502, 5000 U

5'...A↓GATCT...3'
3'...TCTAG↓A...5'

Description

BglII is a Restriction Endonuclease purified from an *Escherichia coli strain* that carries the BglII gene from *Bacillus globigii*. BglII activity is not affected by dam methylation ($G^{m}ATC$) as well as by dcm and CpG methylations. The optimum reaction temperature is at 37°C.

Concentration

10000 U/mL.

Reagents supplied with the enzyme

NZYTech provides BglII with a specific buffer, NZYBuffer A (500 mM Tris-HCl, pH 7.5, 1 M NaCl, 100 mM $MgCl_2$, 10 mM DTT).

Shipping & Storage conditions

Shipped on dry ice. After delivery, product should be stored at -20°C in a non-frost freezer. Minimize exposure of enzyme to temperatures higher than -20 °C. To reduce freeze-thaw cycles, we recommend making small aliquots of the enzyme. BglII will remain stable till the expiry date if stored as specified.

Unit definition

One unit is defined as the amount of this enzyme required to digest completely 1 µg of plasmid DNA in 50 µL of the reaction mixture at 37 °C for one hour.

General protocol

The recommended protocol includes a 10-fold overdigestion, which generally is sufficient to overcome variations than can occur in DNA type, quantity and purity, as well as on frequency of recognition sites. In general, we recommend using 10 units of enzyme to digest 1 µg substrate DNA (or 10-20 units for genomic DNA) in 1 hour at appropriate temperature.

Typical reaction mixture

1. On ice, add the following reaction components into a sterile, nuclease-free microcentrifuge tube (*Note: Enzyme should be the last component added to reaction*):

Substrate DNA	≤ 1 µg
10× NZYBuffer A (*)	2 µL (1x)
BglII	1 µL (10 units)
Sterilized ultrapure water	up to 20 µL

(*) for double digestions, use NZYBuffer U (not provided, Cat. No. MB110)

2. Mix reaction components gently by pipetting or by "flicking" the tube (do not vortex) and spin down.

3. Incubate at 37 °C for one hour.
Note: *In some situations, digestion may be improved by increasing the incubation time.*

Important notes

- *Enzyme should not exceed 10% of total reaction volume.*
- *It is preferable to keep enzyme at -20 °C while working at the bench and just remove it at moment of addition to reaction. Do not keep enzyme on ice for a longer period of time.*
- *Variation on final volume has influence on the reaction. In some situations, small reaction volumes may be beneficial; however, caution should be taken when reducing reaction volume because it may lead to star activity by concentrating glycerol (should not exceed 5-8%), enzyme or salts, as well any contaminant present in the reaction. The recommended final volume is 20 µL but reaction volumes from 10 to 50 µL per µg of substrate DNA can be tested.*
- *Care must be taken during reaction incubation: keep the temperature constant and avoid sample evaporation. This is special critical for long incubation periods (more than 1 hour) and small reaction volumes (less than 15 µL).*

Stopping a reaction

Depending on downstream applications, reaction can be stopped alternatively by:

- Heat Inactivation (20 min. at 65 °C)
- Addition of 20-30 mM EDTA pH 8.0 (*)
- Gel Electrophoresis and Band Excision
- Spin Column DNA Purification
- Phenol-Chloroform Extraction or Ethanol Precipitation

(*) *Note: the chelating property of EDTA may inhibit some downstream applications.*

Activity in NZYTech Buffers

NZYTech Buffers	A	B	C	U
Activity in NZYTech buffers (% of max)	100	(100)	(60)	100

() weak star activity is detected

Quality control assays

Purity

BglII has been determined to be >90% pure as judged by SDS-PAGE followed by BlueSafe staining (Cat. No. MB15201).

DNases assay

10 U of BglII were incubated with 0.2-0.3 µg of pNZY28 for 14-16 hours at 37 °C. No nicking activity was observed following agarose gel electrophoresis.

Functional assay

BglII was tested for performance in a digestion of 1 µg of a recombinant pNZY28 derivative using 10 U, 5 U and 2 U of enzyme. The resulting product was visualized in an agarose gel.

Certificate of Analysis

Test	Result
Enzyme purity	Pass
DNase assay	Pass
Functional assay	Pass

Approved by:

Patrícia Ponte
Senior Manager, Quality Systems

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genes & enzymes

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