MB233_IFU_EN_V2402

DpnII

Catalogue number	Presentation
MB23301	1000 U
MB23302	5000 U

5'... ↓GATC ...3'
3'... CTAG↓ ...5'

Description

NZYtech DpnII Restriction Endonuclease is a high-quality, highly specific endonuclease designed for precise DNA cleavage. This enzyme recognizes and cuts double-stranded DNA at the specific nucleotide sequence displayed above, providing a valuable tool for molecular cloning, DNA mapping, and other genetic manipulation techniques. The enzyme is provided with two distinct buffers to accommodate a variety of applications: NZYBuffer A and NZYBuffer U. NZYBuffer A is optimized for routine applications, ensuring maximal enzyme activity and specificity. NZYBuffer A is the recommended buffer for single-enzyme digestion reactions. NZYBuffer U is a universal buffer designed for digestions involving multiple NZYTech restriction enzymes. In addition, it is a ready-to-load buffer, allowing restriction digestions to be directly loaded into agarose gels without additional preparation. When performing single-enzyme digestions with NZYBuffer A, NZYBuffer U can be used as a loading buffer at a 1x concentration. It is important to note that the use of NZYBuffer U may require slightly longer incubation periods to achieve optimal results due to its universal compatibility.

Shipping & Storage Conditions

This product is shipped at dry ice. After delivery, product should be stored at -85°C to -15°C in a constant temperature freezer. Minimize exposure of enzyme to temperatures higher than -15°C. To reduce freeze-thaw cycles, we recommend making small aliquots of the enzyme. DpnII will remain stable till the expiry date if stored as specified.

Components

	MB23301 (1000 U)		MB23302 (5000 U)	
COMPONENT	**TUBES**	**VOLUME**	**TUBES**	**VOLUME**
DpnII (1000 U)	1	100 µL	5	100 µL
10x NZYBuffer A	1	1 mL	2	1 mL
10x NZYBuffer U	1	1 mL	2	1 mL

Specifications

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.

Inhibition & Inactivation: DpnII is inhibited in the presence of metal chelators (e.g. EDTA), phenol, chloroform, alcohol, detergents or excessive salts. The enzyme's activity can also be inhibited by DNA methylation (see below). The enzyme is heat inactivated at 65°C for 20 min.

Methylation Sensitivity: DpnII activity is blocked by dam methylation.

Optimal Activity temperature: The optimum reaction temperature is at 37°C.

Activity in NZYtech Buffers: DpnII exhibits 100% activity in 10x NZYBuffer A, making it the optimal choice for single-enzyme digestion reactions. In 1x NZYBuffer U, the enzyme may demonstrate slightly reduced activity, which might require adjustments in enzyme quantity or incubation time to achieve optimal results. Despite this, 1x NZYBuffer U is recommended for a streamlined approach in double-digestions due to its compatibility with multiple NZYTech restriction enzymes.

Standard Protocol

Recommendations before starting

- Nucleic acid manipulation: Ensure that DNA is devoid of any potential inhibitors of the enzyme. Additional wash steps during purification are advised. The recommended A230/260 ratio is typically close to 2.0. Achieving this level of purity ensures minimal contamination, which could otherwise interfere with the enzymatic reaction. Store the purified DNA sample properly to prevent degradation. Typically, DNA can be stored at -30°C to -15°C or -80°C to -65°C for long-term stability. We recommend using nuclease-free plasticware and reagents throughout all steps of the procedures.

- Handling instructions: It is preferable to keep the enzyme frozen while working at the bench and only remove it at the moment of addition to the reaction. Do not leave the enzyme on ice for an extended period of time. During setup, the enzyme should be the last component added to the reaction.
- Controls: A negative control without the addition of enzyme is highly recommended for verifying the effectiveness of the digestion process and distinguishing between enzymatic and non-enzymatic sources of DNA fragmentation. This enhances the reliability and interpretability of the experimental results.

Procedure

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.

1. On ice, add the following reaction components into a sterile, nuclease-free microcentrifuge tube
   Notes: *If setting up more than one reaction, prepare a reaction mixture volume 10% greater than the total required for the number of reactions to be performed. If required, include sufficient reactions for the controls. Enzyme should be the last component added to reaction.*

	1 REACTION VOLUME
Substrate DNA	≤ 1 µg
10× NZYBuffer A (1)	2 µL
DpnII	1 µL (10 units)
Nuclease-free water	up to 20 µL

(1) Alternatively, for double digestions or when ready-to-load samples onto agarose gels are required, use 10x NZYBuffer U.

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.*
4. Stop the reaction. Depending on the downstream application, use one of the following inactivation procedures:
   - 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.*
5. When performing digestions with 10X NZYBuffer A, you may add 2 µL of NZYBuffer U to the 20 µL reaction mixture before loading it into an agarose gel. This step ensures optimal loading conditions. Digestions carried out with NZYBuffer U can be directly loaded into agarose gels without additional preparation.

Technical Notes

Reaction Volume: The recommended final volume is 20 µL but reaction volumes from 10 to 50 µL per µg of substrate DNA can be tested. Enzyme should not exceed 10% of total reaction volume. 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.

Incubation Time: 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).

Quality control assays

Purity

DpnII purity is >90% as judged by SDS polyacrylamide gel electrophoresis followed by Coomassie Blue staining.

Nuclease assays

To test for DNase contamination, 1 µg of pNZY28-derivated plasmid DNA are incubated with 10 U of enzyme for 14-16 h at 37 °C. Following incubation, the nucleic acid is visualized on a GreenSafe-stained agarose gel. There must be no visible nicking or cutting of the nucleic acid.

Functional assay

DpnII 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.