Speedy NcoI

Catalogue number	Presentation
MB10001	50 reactions
MB10002	250 reactions

5'...C↓CATGG...3'
3'...GGTAC↓C...5'

Description

Speedy NcoI is a in ultra-fast version of the conventional NcoI restriction endonuclease. NZYtech's Speedy restriction enzymes represent a new generation of DNA-modifying enzymes designed for rapid DNA digestion, thereby saving time and effort. These enzymes recognize and cut double-stranded DNA at the specific nucleotide sequence displayed above within 5-15 minutes using the provided reaction buffers. The abbreviated incubation period is advantageous for mitigating star activity, ensuring high specificity and efficiency in your molecular biology workflows. NZYtech supplies these enzymes with two reaction buffers: NZYBuffer B and NZYBuffer U. NZYBuffer B is optimized for routine applications, ensuring maximal enzyme activity and specificity. NZYBuffer B 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 B, 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. Speedy NcoI will remain stable till the expiry date if stored as specified.

Components

	MB10001 (50 reactions)		MB10002 (250 reactions)	
COMPONENT	**TUBES**	**VOLUME**	**TUBES**	**VOLUME**
Speedy NcoI (50 reactions)	1	50 µL	5	50 µL
10x NZYBuffer B	1	1 mL	1	1 mL
10x NZYBuffer U	1	1 mL	1	1 mL

Specifications

Activity definition: One µL of enzyme can completely digest up to 1 µg of DNA in 5-15 min.

Inhibition & Inactivation: Speedy NcoI is inhibited in the presence of metal chelators (e.g. EDTA), phenol, chloroform, alcohol, detergents or excessive salts. The enzyme's activity is not inhibited by DNA methylation (see below). The enzyme is heat inactivated at 65°C for 20 min.

Methylation Sensitivity: Speedy NcoI activity is not affected by dam methylation, dcm methylation or CpG methylation.

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

Activity in NZYtech Buffers: Speedy NcoI exhibits 100% activity in 10x NZYBuffer B, 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

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 B (1)	2 µL
Speedy NcoI	1 µL
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 5-15 minutes.

   Note: *The enzyme is is fast enough to allow DNA digestion in as little as 5 minutes. However, in certain cases (e.g., for longer or complex subtrate or when multiple restriction sites need to be cleaved), increasing the incubation time up to 15-20 minutes may enhance complete digestion.*

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 B, 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

Enzyme: The recommended volume of enzyme in standard reactions involving up to 1 µg of substrate in 20 µL is 1 µL. Using a lower volume of enzyme may result in incomplete digestions.

Reaction Volume: The recommended final volume is 20 µL, but adjustments may be necessary in some cases. Please note that reducing the reaction volume may lead to star activity due to enzyme concentration.

Quality control assays

Purity

Speedy NcoI 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 1 µL 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

Speedy NcoI was tested for performance in a digestion of 1 µg of a recombinant pNZY28 derivative using 1 µL of enzyme in a 20 µL-reaction. The resulting product was visualized in an agarose gel, against the negative control without enzyme.

For life science research only. Not for use in diagnostic procedures.

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