MB242_IFU_EN_V2401

Speedy MboI

Catalogue number	Presentation
MB24201	100 reactions
MB24202	500 reactions

5'... ↓GATC ...3'
3'... CTAG↓ ...5'

Description

NZYtech's Speedy restriction enzymes represent a new generation of DNA-modifying enzymes designed for rapid DNA digestion, thereby saving time and effort. All Speedy enzymes exhibit 100% activity when used with the NZYSpeedy Buffers and can effectively digest DNA within 5-15 minutes. This abbreviated incubation period is advantageous for mitigating star activity. The NZYSpeedy Buffers are universally compatible, enabling the use of any combination of NZYSpeedy restriction enzymes. This feature is particularly advantageous for conducting double or multiple digestions, eliminating the necessity for sequential digestions. Furthermore, the NZYSpeedy Buffer Orange allows ready gel loading post-digestion. Speedy MboI is a in ultra-fast version of the conventional MboI restriction endonuclease.

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 MboI will remain stable till the expiry date if stored as specified.

Components

	MB24201 (100 reactions)		MB24202 (500 reactions)	
COMPONENT	**TUBES**	**VOLUME**	**TUBES**	**VOLUME**
Speedy MboI (100 reactions)	1	100 µL	5	100 µL
10x NZYSpeedyBuffer Colourless	1	200 µL	1	1 mL
10x NZYSpeedyBuffer Orange	1	200 µL	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 MboI 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: Speedy MboI activity is blocked by dam methylation and impaired by overlapping CpG methylation.

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

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× NZYSpeedy Buffer (1)	2 µL
Speedy MboI	1 µL
Nuclease-free water	up to 20 µL

(1) Depending on the applications, choose 10x NZYSpeedyBuffer Colourless or 10x NZYSpeedyBuffer Orange. Refer to "Technical Notes" below.

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.*

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 MboI 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 MboI 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.