

mRNex® Precise restriction enzyme XbaI

Cat. no. LD101006

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Product Information

Product name	mRNex® Precise restriction enzyme XbaI
Cat.No.	LD101006
Size	500T
Storage and shipping	Dry ice transportation. Store at -20°C, valid for two years.
Recognition site	5'-T↓CTAGA-3' 3'-AGATC↑T-5'
Recommended reaction cons	1× SharpSeq Buffer; optimal for incubation at 37°C
Enzyme activity	20 U/μL
Inactivation conditions	Inactivate at 80°C for 20 min.

2. Product Description

The SharpSeq series of endonucleases are rapid restriction endonucleases that can accurately complete DNA cleavage within 5 ~ 15 minutes using genetic engineering recombinant technology. They are suitable for quickly cleaving plasmid DNA, PCR products, genomic DNA, and more. The SharpSeq series of rapid endonucleases use a common cutting buffer, simplifying the enzyme digestion reaction system. Additionally, they exhibit excellent enzyme activity redundancy, making it easy to handle excess substrate or difficult templates during cleavage.

3. Components

Cat No.	Component	LD101006 (500 T)
LD101006-A	SharpSeq XbaI	500μL
LD101006-B	10×SharpSeq Buffer	3×1mL
LD101006-C	10×SharpSeq Color Buffer*	3×1mL

*10 × SharpSeq Color Buffer includes red and yellow tracking dyes, which allow the product to be directly used for gel electrophoresis. The red dye in SharpSeq Color Buffer migrates at a rate similar to that of a 2500 bp double-stranded DNA fragment in a 1% agarose gel, while the yellow dye migrates at a rate similar to that of a 10 bp double-stranded DNA fragment in a 1% agarose gel.

4. Notes

4.1. 3h incubation did not show star activity (refer to Introductions for use, 6), delayed digestion may result in star activity.

4.2. Affected by Dam methylation, the sequence may overlap, leading to cleavage blockage.

4.3. For your safety and health, please wear lab coats and disposable gloves.

4.4. For research use only!

5. Instructions for Use

5.1. DNA quick digestion procedure

5.1.1. prepare the reaction mixture according to the suggested order of addition (on ice):

Component	Plasmid DNA	PCR product	Genomic DNA
ddH ₂ O	15 µL	16 µL	30 µL
10 × SharpSeq Buffer or 10 × SharpSeq Color Buffer	2 µL	3 µL	5 µL
Substrate DNA	2 µL (~1 µL)	10 µL (~0.2 µL)	10 µL (~5 µL)
SharpSeq XbaI	1 µL	1 µL	5 µL
Total	20 µL	30 µL	50 µL

*This system refers to purified PCR products. Unpurified PCR products have a certain ionic strength, and the amount of 10×SharpSeq Buffer can be appropriately reduced to 2 µL. If cloning or other experiments are performed in the next step, the PCR product must be purified before digestion.

5.1.2. Gently pipette or tap the tube to mix (do not vortex), then briefly centrifuge to collect droplets from the tube walls.

5.1.3. Incubate at 37°C for 15 minutes (plasmid), or 15–30 minutes (PCR product), or 30–60 minutes (genomic DNA).

5.1.4. Incubate at 80°C for 20 minutes to inactivate the enzyme and stop the reaction (optional).

5.1.5. If using SharpSeq Color Buffer for the digestion reaction, the resulting product can be directly loaded for electrophoresis.

5.2. Double or Multiple Enzyme Digestion

5.2.1. The amount of each restriction enzyme should be 1 µL, and the reaction system can be appropriately scaled up as needed.

5.2.2. The total volume of all restriction enzymes should not exceed 1/10 of the total reaction volume.

5.2.3. If the selected restriction enzymes have different optimal reaction temperatures, digestion should first be carried out with the enzyme having the lower optimal temperature, and then the enzyme with the higher optimal temperature should be added, followed by incubation at the higher temperature.

5.3. Plasmid amplification reaction system

Component	Volume(20 µL)	Volume(20 µL)	Volume(50 µL) *
DNA	1µg	2µg	5µg
SharpSeq XbaI	1µg	2µg	5µg
10×SharpSeq Buffer or 10×SharpSeq Color Buffer	2µg	2µg	5µg
Total	20µg	20µg	50µg

* If the total reaction volume is greater than 20 µL, a water bath, metal bath, or sand bath can be used, and the incubation time should be increased.

5.4. The number of recognition sites in different DNAs

λDNA	ΦX174	pBR322	pUC57	pUC18/19	SV40	M13mp18/19	Adeno2
1	0	0	1	1	0	1	5

5.5. The effect of methylation modifications

Dam	Dcm	CpG	EcoKI	EcoBI
Potentially causing overlap or cleavage blockage	no effect	no effect	no effect	no effect

5.6. Activity in different reaction buffers*

Reaction buffer	SharpSeq Buffer	Thermo Scientific Fast Digest Buffer	NEB CutSmart Buffer	Takara QuickCut TM Buffer
Activity	100%	50%	100%	100%

* The activity data were obtained from assays conducted under Medino Biotech's standard restriction enzyme reaction system.