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# **BstEII**

### 1 Contents

Component	HY-KE7009-100T			
BstE II	100 μL			
10× Buffer	1 mL			
10× Color Buffer	1 mL			

### 2 Introduction

BstE II is a restriction enzyme for rapid DNA digestion, including plasmid, genomic DNA as well as PCR products. Isoschizomers: BstP I, Eco91 I, Eco965 I, PspE I.

BstE II is 100% active in 10× Buffer and 10× Color Buffer. 10× Color Buffer includes a density reagent along with red and yellow tracking dyes that allow for direct loading of the reaction mixtures on a gel. The red dye of the Color Buffer migrates with 2500 bp DNA fragments in a 1% agarose gel and the yellow dye of the Color Buffer migrates faster than 10 bp DNA fragments in a 1% agarose gel.

Cleavage site

5'...G T N A C C...3'

3'...C C A N T G<sub>1</sub>G...5'

# 3 General Protocol

- 1. Fast Digestion of Different DNA
- 1.1 Combine the following reaction components on ice in the order indicated:

	Plasmid DNA	PCR product	Genomic DNA
ddH <sub>2</sub> O	15 μL	16 μL	30 µL
10× Buffer or 10× Color Buffer	2 μL	3 µL	5 μL
DNA	2 μL (up to 1 μg)	10 μL (~ 0.2 μg)	10 μL (5 μg)
BstE II	1 μL	1 μL	5 μL
Total	20 μL	30 μL	50 μL

Note: When PCR product will be used for cloning, it is recommended to purify PCR product prior digestion.

- 1.2 Mix gently and spin down.
- 1.3 Incubate at 37°C for 15 min (Plasmid DNA), or for 15-30 min (PCR product), or for 30-60 min (Genomic DNA).
- 1.4 (Optional) Inactivate the enzyme by heating for 20 min at 80°C.
- 1.5 If 10× Color Buffer was used in the reaction, load an aliquot of the reaction mixture directly on a gel.

#### 2. Scaling up Plasmid DNA Digestion Reaction

DNA	1 μg	2 μg	3 μg	4 μg	5 μg
BstE II	1 μL	2 μL	3 μL	4 μL	5 μL
10× Buffer or 10× Color Buffer	2 μL	2 μL	3 μL	4 μL	5 μL
Total	20 μL	20 µL	30 µL	40 μL	50 μL

Note: Increase the incubation time by 3-5 min if the total reaction volume exceeds 20 µL. It is recommended to use heat block or water thermostat.

# 4 Number of Recognition Sites in DNA

λDNA	ФХ174	pBR322	pUC57	pUC18/19	SV40	M13mp18/19	Adeno2
13	0	0	0	0	0	0	10

# 5 Methylation Effects on Digestion

Dam	Dcm	CpG	EcoKI	EcoBI
no effect	no effect	no effect	blocked	blocked

### 6 Storage

-20°C, 2 years.

# 7 Precautions

- 1. The combined volume of the enzymes in the reaction mixture should not exceed 1/10 of the total reaction volume.
- 2. Use 1  $\mu L$  of each enzyme and scale up the reaction conditions appropriately.
- 3. If the enzymes require different reaction temperatures, start with the enzyme that requires a lower temperature, then add the second enzyme and incubate at the higher temperature.
- 4. This product is for R&D use only, not for drug, household, or other uses.
- 5. For your safety and health, please wear a lab coat and disposable gloves to operate.