Version: M17L05V1.0

BsmBI

Cat. No. : RK21173

Product Components

Components	Component Number	200 U	1,000 U
BsmBI (10,000 U/mL)	RM21673	20 μL	100 μL
10X BsmBI Buffer	RM20111	1 mL	1 mL

Product Description

Restriction Site

5'...C G T C T C (N)$_1$▼...3'

3'...G C A G A G (N)$_5$▲...5'

Unit Definition

One unit is defined as the amount of enzyme required to digest 1 μg of λDNA in 1 hour at 55°C in a total reaction volume of 50 μL.

Storage

-20°C

Reaction Conditions

1X BsmBI Buffer, incubate at 55°C.

Heat Inactivation

80°C for 20 min.

Instructions

Recommended Protocol for Digestion

Components	Volume
ddH_2O	Up to 50 μL
10X BsmBI Buffer	5 μL
PCR fragment or donor vector	1 μg
BsmBI*	1 μL

* Note: In general, we recommend 5-10 units of BsmBI per μg Plasmid DNA, and 10-20 units for genomic DNA in a 1 hour digest.

- Mix components by pipetting the reaction mixture up and down, or by "flicking" the reaction tube. Follow with a quick ("touch") spin-down in a microcentrifuge. Do not vortex the reaction.
- Incubate at 55°C for 1 hr.
- Inactivated at 80°C for 20 min. (Optional)

Note

1. Enzyme

- Keep on ice when not in the freezer.
- Should be the last component added to reaction.

2. DNA

- Should be free of contaminants such as phenol, chloroform, alcohol, EDTA, detergents or excessive salts. Extra wash steps during purification are recommended.
- Methylation of DNA can inhibit digestion with certain enzymes.
- Methylation Sensitivity

Dam	not sensitive
Dcm	not sensitive
CpG	may overlap, cleavage impaired
EcoKI	not sensitive
EcoBI	may overlap, effect not determined

- Number of Recognition Sites in DNA

pUC57	pUC18/19	pBR322	λDNA
2	2	1	14

3. Buffer

- Use at a 1X concentration.

4. Reaction Volume

- A 50 µL reaction volume is recommended for digestion of 1 µg of substrate.
- Enzyme volume should not exceed 10% of the total reaction volume to prevent star activity due to excess glycerol.
- Additives in the restriction enzyme storage buffer (e.g., glycerol, salt) as well as contaminants found in the substrate solution (e.g., salt, EDTA, or alcohol) can be problematic in smaller reaction volumes. The following guidelines can be used for techniques that require smaller reaction volumes.

Reaction System	Enzyme Amount*	DNA	10X BsmBI Buffer
10 µL**	1 U	0.1 µg	1 µL
25 µL	5 U	0.5 µg	2.5 µL
50 µL	10 U	1 µg	5 µL

* Note: Restriction Enzymes should be diluted when smaller amounts are needed. ** Note: 10 µL rxns should not be incubated for longer than 1 hour to avoid evaporation.

5. Incubation Time

- Incubation time is typically 1 hour.
- Can often be decreased by using an excess of enzyme, or by using one of our "Quick Cut" restriction enzymes.

6. Recommended Protocol for One-step ligation reactions

Components	Amount
Nuclease-free water	to 20 µL
Inserts*	0.1 pmol
Plasmid	0.05 pmol
10x T4 DNA ligase Reaction buffer	2 µL
T4 DNA Ligase (High Conc.) (RK21500)	1 µL
BsmBI	1 µL

* Note: When multiple inserts are present, 0.1 pmol is invested for each inserts.

- **Assembly Protocol for 1 Insert**

Temperature	Time	Cycle
42℃	10 min	1
60℃	5 min	1

- **Assembly Protocol for 2+ Inserts**

Temperature	Time	Cycle
42℃	1 min	30
16℃	1 min	
60℃	5 min	1

7. Control Reactions

If you are having difficulty cleaving your DNA substrate, we recommend the following control reactions:

- Control DNA (DNA with multiple known sites for the enzyme, e.g. lambda or pUC19 DNA) with restriction enzyme to test enzyme viability.
- If the control DNA is cleaved and the experimental DNA resists cleavage, the two DNAs can be mixed to determine if an inhibitor is present in the experimental sample. If an inhibitor (often salt, EDTA or phenol) is present, the control DNA will not cut after mixing.