

MBRE002

BamH I

Components

| Reagents provided | MBRE002 | | | |
|-------------------|-----------|-----------|------------|------------|
| | 250 Units | 500 Units | 1000 Units | 5000 Units |
| BamH I | 12.5 µl | 25 µl | 50 µl | 250 µl |
| 10X HiBuffer DB | 100 µl | 200 µl | 400 µl | 2 ml |
| Diluent E Buffer | 50 µl | 100 µl | 200 µl | 1 ml |

NOTE: BSA included in all Reaction Buffer

Source: A E.coli strain that carries the BamH I gene from *Bacillus amyloliquefaciens*

Recognition Sequence:



Concentration: 20 U/µl

Unit Definition:

1 u is defined as the amount of enzyme that is required to digest 1µg of DNA in 1 hour at 37°C in 50 µl of assay buffer.

| Enzyme | Optimum reaction temperature (°C) | Thermal Inactivation (°C) | % activity of Buffers | | | | |
|--------|-----------------------------------|---------------------------|-----------------------|----|----|----|----|
| | | | H1 | H2 | H3 | H4 | H5 |
| BamH I | 37 | 65 | 75 | 75 | 50 | 75 | 75 |

Reaction Buffer:

10X HiBuffer DB:

25mM Tris -acetate (pH 7.6 at 30°C), 10 mM Mg-acetate, 100 mM K-acetate, 7mM 2-mercaptoethanol, 50 µg /ml BSA.

NOTE: Incubate at 37°C

Storage Buffer:

10mM Tris -HCl (pH 7.6), 50 mM NaCl, 0.1 mM EDTA, 200 µg/ml BSA, 1 mM DTT, 50% Glycerol. Store at -20°C

Quality Control Assays:

Ligation / Recutting Assay:

After 40 -fold over digestion with BamH I, more than 90% of the DNA fragments can be ligated and recut.

Over digestion assay:

An unaltered banding pattern was observed after 1µg of DNA was digested with 40U of BamH I for 16 hours at 37°C.

Example of Digestion Reaction:

- Enzyme concentration : 1 Unit
- Lambda DNA (0.3 µg /µl) : 3.33 µl (1 µg DNA)
- 10X HiBuffer DB : 5 µl
- Nuclease free water : upto 50 µl

Note:

- Total reaction volume is dependent on the experiment
- The amount of enzyme to be used is dependent on the DNA template
- For plasmid DNA, 5-10X more enzyme is required
- High enzyme concentration may result in **Star activity**

Storage conditions: BamH I should be stored at -20°C.