MBRE007 **Hae III**

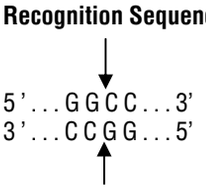
Components

Reagents provided	MBRE007			
	250 Units	500 Units	1000 Units	5000 Units
Hae III	31.25 µl	62.5 µl	125 µl	625 µl
10X HiBuffer H4	250 µl	500 µl	1 ml	5 ml
10X HiBuffer DB	250 µl	500 µl	1 ml	5 ml
Diluent E Buffer	125 µl	250 µl	500 µl	2.5 ml

NOTE: BSA included in all Reaction Buffer

Source: A *E.coli* strain that carries the Hae III gene from *Haemophilus aegyptius*

Recognition Sequence:



Concentration: 8 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 50µl of assay buffer.

Enzyme	Optimum reaction temperature (°C)	Thermal Inactivation (°C)	% activity of Buffers				
			H1	H2	H3	H4	H5
Hae III	37	65	75	75	10	100	100

Reaction Buffer:

10X HiBuffer H4:
10mM Tris –HCl (pH 8.5 at 30°C), 10 mM $MgCl_2$, 100 mM KCl and 100 µg /ml BSA.
NOTE: Incubate at 37°C

Storage Buffer:
10mM Tris-HCl (pH 7.5), 300mM NaCl, 0.1mM EDTA, 7mM 2-mercaptoethanol, 200µg/ml BSA and 50% glycerol. Store at –20°C .
NOTE: 10X HiBuffer DB is provided for double digestion.

Quality Control Assays:
Ligation / Recutting Assay:
After 8 -fold over digestion with Hae III, 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 16U of Hae III for 16 hours at 37°C.

ISO 9001-2008 CERTIFIED

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Ideal Digestion conditions:

- Enzyme concentration : 1 Unit
- Lambda DNA (0.3 µg /µl) : 3.33 µl (1 µg DNA)
- 10X HiBuffer H4 : 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: Hae III should be stored at -20°C.

MBRE007_/0611 MBRE007-02