MBRE003 **Hind III**

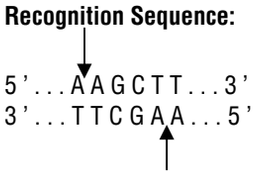
Components

Reagents provided	MBRE003			
	250 Units	500 Units	1000 Units	5000 Units
Hind III	12.5 µl	25 µl	50 µl	250 µl
10X HiBuffer H2	100 µl	200 µl	400 µl	2ml
10X HiBuffer DB	100 µl	200 µl	400 µl	2ml
Diluent E Buffer	50 µl	100 µl	200 µl	1ml

NOTE: BSA included in all Reaction Buffer

Source: A *E.coli* strain that carries the Hind III gene from *Haemophilus influenzae*

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 50µl of assay buffer.

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

Reaction Buffer:

10X HiBuffer H2:
10mM Tris -HCl (pH 7.5 at 30°C), 10mM $MgCl_2$, 50mM NaCl and 100µg/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 20-fold over digestion with Hind III, more than 90% of the DNA fragments can be ligated and recut.

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Over digestion Assay:
An unaltered banding pattern was observed after 1µg of DNA was digested with 40U of Hind III for 16 hours at 37^{O}C.

Example of Digestion conditions:

- Enzyme concentration : 1 Unit
- Lambda DNA 0.3 µg/µl : 3.33 µl (1 µg DNA)
- 10X HiBuffer H2 : 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: Hind III should be stored at -20°C.

MBRE003_/0611 MBRE003-02