MBRE005 **Not I**

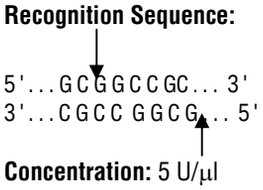
Components

Reagents provided	MBRE005			
	250 Units	500 Units	1000 Units	5000 Units
Not I	50 µl	100 µl	200 µl	1 ml
10X HiBuffer H5	25 µl	50 µl	0.1ml	0.5ml
10X HiBuffer DB	25 µl	50 µl	0.1 ml	0.5 ml
Diluent E Buffer	25 µl	50 µl	0.1 ml	0.5 ml

NOTE: BSA included in all Reaction Buffer

Source: A *E.coli* strain that carries the Not I gene from *Nocardia otitidis*

Recognition Sequence:



Concentration: 5 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
Not I	37	65	50	75	75	75	100

Reaction Buffer:

10X HiBuffer H5:
30mM Tris-acetate (pH 7.9 at 30°C), 10mM Mg-acetate, 60mM K-acetate 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 5-fold over digestion with Not 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 10U of Not I for 16 hours at 37°C.

ISO 9001-2008 CERTIFIED

Registered Office :
23, Vadhani Industrial Estate,LBS Marg,
Mumbai - 400 086, India.
Tel. : (022) 4017 9797 / 2500 1607
Fax : (022) 2500 2286

Commercial Office
A-516, Swastik Disha Business Park,
Via Vadhani Indl. Est., LBS Marg,
Mumbai - 400 086, India
Tel: 00-91-22-6147 1919
Fax: 6147 1920, 2500 5764
Email : info@himedialabs.com
Web : www.himedialabs.com

Example of Digestion conditions:

- Enzyme concentration : 1 Unit
- Lambda 0.3 μg/μl : 3.33 μl (1 μg DNA)
- 10X HiBuffer H5 : 2.5 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: Not I should be stored at -20°C.

MBRE005_/0611 MBRE005-02