MBRE001 **EcoR I**

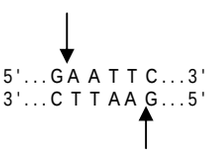
Components

Reagents provided	MBRE001			
	250 Units	500 Units	1000 Units	5000 Units
EcoR I	12.5 µl	25 µl	50 µl	250 µl
10X HiBuffer EcoR I	100 µl	200 µl	400 µl	2 ml
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 EcoR I gene from *Escherichia coli*

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^{o}C in 50µl of assay buffer.

Enzyme	Optimum reaction temperature (^{0}C)	Thermal Inactivation (^{0}C)	% activity of Buffers				
			H1	H2	H3	H4	H5
EcoR I	37	65	50	50	100	100	50

Reaction Buffer:

10X HiBuffer EcoR I:
50mM Tris -HCl (pH 7.5 at 30°C), 10 mM $MgCl_2$, 100 mM NaCl , 0 .0 2 % triton X – 100 , 0 .1 mg /ml BSA.
NOTE: Incubate at 37^{o}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^{o}C

NOTE: 10X HiBuffer DB is provided for double digestion.

Quality Control Assays:

Ligation / Recutting Assay:
After 18 -fold over digestion with EcoR I, more than 95% of the DNA fragments can be ligated and recut.

ISO 9001-2008 CERTIFIED

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Over digestion assay:
An unaltered banding pattern was observed after 1µg of DNA was digested with 36U of EcoR I for 16 hours at 37^{O}C.

Example of Digestion Reaction:

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

MBRE001_/0611 MBRE001-02