MBRE009 **EcoR V**

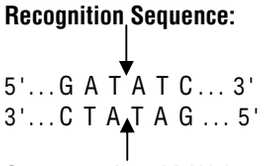
Components

Reagents provided	MBRE009			
	250 Units	500 Units	1000 Units	5000 Units
EcoR V	12.5 µl	25 µl	50 µl	250 µl
10X HiBuffer EcoR V	25 µl	50 µl	0.1 ml	0.5 ml
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 EcoR V 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 1□g of DNA in 1 hour at 37^{0}C in 50□l of assay buffer.

Enzyme	Optimum reaction temperature (^{0}C)	Thermal Inactivation (^{0}C)	% activity of Buffers				
			H1	H2	H3	H4	H5
EcoR V	37	None	0	0	100	75	0

Reaction Buffer:

10X HiBuffer EcoR V:
10mM Tris-HCl (pH 8.5 at 30^{0}C), 10mM Mg-Cl_2, 100mM NaCl, and 100 µg/ml BSA.
Note: Incubate at 37^{0}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^{0}C .
NOTE: 10X HiBuffer DB is provided for double digestion.

Quality Control Assays:

Ligation / Recutting Assay:
After 20-fold over digestion with EcoR V, 80% 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 EcoR V for 16 hours at 37^{0}C.

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Example of Digestion conditions:

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

MBRE009_/0611 MBRE009-02