MBRE016 **Xba I**

Components

Reagents provided	MBRE016			
	250 Units	500 Units	1000 Units	5000 Units
Xba I	12.5 µl	25 µl	50 µl	250 µl
10X HiBuffer H5	312.5 µl	625 µl	1.25 ml	6.25 ml
10X HiBuffer DB	312.5 µl	625 µl	1.25 ml	6.25 ml
Diluent E Buffer	162.5 µl	325 µl	650µl	3.25 ml

NOTE: BSA included in all Reaction Buffer

Source: A *E.coli* strain that carries the Xba I gene from *Xanthomonas badrii*

Recognition Sequence:

5'...TCTAGA...3'
3'...AGATCT...5'

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^{0}C in 50µl of assay buffer.

Enzyme	Optimum reaction temperature (^{0}C)	Thermal Inactivation (^{0}C)	% activity of Buffers				
			H1	H2	H3	H4	H5
Xba I	37	65	10	75	75	10	100

Reaction Buffer:

10X HiBuffer H5:
30mM Tris –acetate (pH 7.9 at 30°C), 10 mM Mg-acetate, 60 mM K-acetate 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°C .
NOTE: 10X HiBuffer DB is provided for double digestion.

Quality Control Assays:

Ligation / Recutting Assay :
After 20 -fold overdigestion with Xba 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 40U of Xba I for 16

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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 H5 : 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: Xba I should be stored at -20°C.