MBRE012 **Sac I**

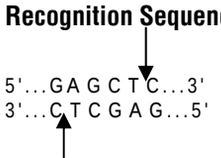
Components

Reagents provided	MBRE012			
	250 Units	500 Units	1000 Units	5000 Units
Sac I	6.25 µl	12.5 µl	25 µl	125 µl
10X HiBuffer H2	500 µl	1 ml	2 ml	10 ml
10X HiBuffer DB	500 µl	1 ml	2 ml	10 ml
Diluent E Buffer	250 µl	500 µl	1 ml	5 ml

NOTE: BSA included in all Reaction Buffer

Source: A *E.coli* strain that carries the Sac I gene from *Streptomyces achromogenes*

Recognition Sequence:



Concentration: 40 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
Sac I	37	65	100	100	75	100	100

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^{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 40-fold over digestion with Sac I, more than 95% 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 80U of Sac I for 16 hours at 37^{O}C.

Example of Digestion conditions:

- Enzyme concentration : 1 Unit
- Lambda (Hind III digest)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: Sac I should be stored at -20°C.

MBRE012_/00611 MBRE012-02