MBRE014 **Sph I**

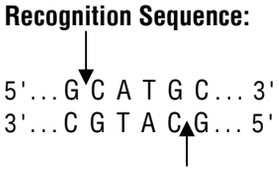
Components

Reagents provided	MBRE014			
	250 Units	500 Units	1000 Units	5000 Units
Sph I	50 µl	100 µl	200 µl	1 ml
10X HiBuffer DB	1.25 ml	2.5 ml	5 ml	25 ml
Diluent E Buffer	625 µl	1.25 ml	2.5 ml	12.5 ml

NOTE: BSA included in all Reaction Buffer

Source: A *E.coli* strain that carries the Sph I gene from *Streptomyces phaeochromogenes*

Recognition Sequence:



Concentration: 5 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
Sph I	37	65	75	75	50	75	75

Reaction Buffer:

10X HiBuffer DB:
25mM Tris –acetate (pH 7.6 at 30°C), 10 mM Mg-acetate, 100 mM K-acetate, 7mM 2-mercaptoethanol and 50 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 10-fold over digestion with Sph 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 20U of Sph I for 16 hours at 37^{0}C.

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Example of Digestion conditions:

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

MBRE014_/0611 MBRE014-02