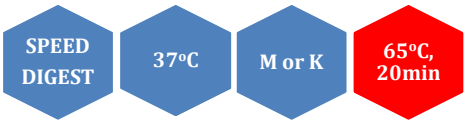
Sph I



5’ ···GCATG▼C···3’
3’ ···C▲GTACG···5’

SphI is a restriction enzyme purified from *Streptomyces phaeochromogenes*.

Catalogue No — *137-1, 500 U*
137-2, 3x500 U

Concentration — *10-12u/µl and 40-60u/µl**

**Add an H to cat.# to order the high concentration*

Reagents supplied: 10x M and 10x K buffer

Unit substrate: Lambda DNA.

Unit calculation assay conditions: 50 mM NaCl, 10 mM Tris-HCl (pH 7.9 @ 25°C), 10 mM $MgCl_2$, 1 mM dithiothreitol, 100 µg/ml BSA. Incubate at 37°C.

Absence of contaminants: 50 units of *Sph*I do not produce any unspecific cleavage products after 16 hrs incubation with 1 µg of λ DNA at 37°C. After 10-fold overdigestion with *Sph*I, greater than 98% of the DNA fragments can be ligated and recut with this enzyme.

Storage buffer: 100 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 400 µg/ml BSA and 50% glycerol. Store at -20°C.

Heat inactivation: 65°C for 20 minutes.

Methylation Sensitivity:
dam methylation: Not sensitive
dcm methylation: Not sensitive
CpG methylation: Not sensitive

Percent Activity in MINOTECH Buffers

L	**M**	H	SH	A	**K**
75-100	**100**	50	50	50	**100**

General reaction mixture:

10U SphI	1µl
10x M or K buffer *	2µl
DNA substrate	<1µg
Sterile ultrapure water	Up to 20 µl

Incubate for 15 min at 37°C

**In the case of M buffer we recommend the addition of BSA to a final concentration of 100 µg/ml.*

Frequency of Cutting

λ	Ad-2	Φx174	pUC18	M13mp18	pBR322
6	8	0	1	1	1

Lambda DNA 0.7 % agarose

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