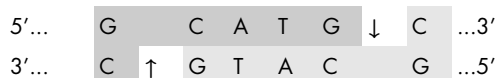



Sph I

 Isoschizomers: *Bbu* I, *Pae* I, *Spa*HI

Cat. No.	Amount
EN-137S	250 Units
EN-137L	5 x 250 Units



Unit Definition: One unit is the amount of enzyme required to completely digest 1 μ g of Lambda DNA (1 site) in 1 hour in a total reaction volume of 50 μ l. Enzyme activity was determined in the recommended reaction buffer.

For in vitro use only!

Shipping: shipped on blue ice

Storage Conditions: store at -20 °C

Additional Storage Conditions: avoid freeze/thaw cycles

Shelf Life: 12 months

Form: liquid (Supplied in 10 mM Tris-HCl pH 7.4, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 400 μ g/ml BSA and 50 % [v/v] glycerol)

Concentration: 10 units/ μ l

Source: *Streptomyces phaeochromogenes*

Supplied with: 10x Universal Buffer (UB)

Recommended 50 μ l assay

10 μ l*	10x Universal Buffer (UB)
1 μ g	pure DNA ¹ or PCR product ²
5 units	enzyme
fill up to 50 μ l	PCR grade water

Optimum buffer condition for restriction is 2x UB

¹ Supercoiled or high molecular weight DNA (e.g. plant genomic DNA) may require longer incubation time or higher amount of enzyme.

² Some enzymes may require additional DNA bases flanking the restriction site for complete digestion.

Protocol:

- The enzyme should not exceed 10 % of total reaction volume.
- Add enzyme as last component. Mix components well before adding enzyme. After enzyme addition, mix gently by pipetting. Do not vortex.
- Incubate 60 min. at 37 °C.
- Stop reaction by alternatively:
 - Addition of 2.1 μ l EDTA pH 8.0 [0.5 M], final 20 mM
 - Heat Inactivation (20 min. at 65 °C)
 - Spin Column DNA Purification (e.g. PCR Purification Kit, Cat.-No. PP-201S/L)
 - Gel Electrophoresis and Single Band Excision (e.g. Agarose Gel Extraction Kit, Cat.-No. PP-202S/L)
 - Phenol-Chloroform Extraction or Ethanol Precipitation.

Double Digestion - Buffer Compatibility:

B1 - 75-100 % Relative Activity

B2 - 100 % Relative Activity

B3 - 50 % Relative Activity

B4 - 50 % Relative Activity

B5 - 50 % Relative Activity

2x UB - 100 % Relative Activity (recommended)

Please note that the optimum digestion condition for this enzyme is 2x UB. Within the Universal Buffer (UB) system, the most majority of our enzymes display 100% Relative Activity in 1x UB and only few either in 0.5x or 2x UB. If optimum condition for second enzyme is different than the recommended for the first enzyme, we suggest carrying out first the restriction at the higher recommended concentration of UB and dilute the reaction volume to the adequate UB concentration for further proceeding with the second restriction.

DATA SHEET



Sph I

Isoschizomers: *Bbu* I, *Pae* I, *Spa*HI

Reaction Enzymes Buffer Guide:

Buffer 1	10 × B1	100 mM 100 mM 10 mM 1000 µg/ml	Tris-HCl (pH 7.9, 25°C) MgCl ₂ Dithiothreitol BSA
Buffer 2	10 × B2	100 mM 100 mM 500 mM 10 mM 1000 µg/ml	Tris-HCl (pH 7.9, 25°C) MgCl ₂ NaCl Dithiothreitol BSA
Buffer 3	10 × B3	500 mM 100 mM 1000 mM 10 mM 1000 µg/ml	Tris-HCl (pH 7.9, 25°C) MgCl ₂ NaCl Dithiothreitol BSA
Buffer 4	10 × B4	100 mM 100 mM 1500 mM 10 mM 1000 µg/ml	Tris-HCl (pH 7.9, 25°C) MgCl ₂ NaCl Dithiothreitol BSA
Buffer 5	10 × B5	200 mM 100 mM 500 mM 10 mM 1000 µg/ml	Tris-acetate (pH 7.9, 25°C) Mg-acetate K-acetate Dithiothreitol BSA

DNA Methylation:

No Inhibition: dam, dcm, CpG

Quality Control:

All preparations are assayed for contaminating endonuclease, 3'-exonuclease, 5' exonuclease/ 5' phosphatase, as well as nonspecific single- and doublestranded DNase activities.

Reaction Buffer Compatibility:

Our restriction enzymes are fully compatible to restrictases and buffer systems from other manufacturers and can be used along in double digestions. To obtain best results, consult the corresponding manuals of all involved products.

Ligation and recutting:

After 10-fold overdigestion with *Sph* I, >98% of the DNA fragments can be ligated and recut with this enzyme.