



Restriction Enzyme

Sph I

II 37 65

| Cat.# | Size | Conc. |
|---------|-----------|------------------|
| FG-SphI | 600 units | 5 units/ μ l |

Store at -20°C

Supplied with: 10X FastGene® Buffer II (FG-REB2)
10X FastGene® FastCut Buffer (FG-REBHF)
6X DNA Loading Buffer
Sterile water

Recognition site



For Research Use Only. Not for use in diagnostic procedures.

ISO9001

Source: *Streptomyces phaeochromogenes*

Reaction conditions

1X FastGene® Buffer II, 37°C
1X FastGene® FastCut Buffer, 37°C

FastGene® FastCut Buffer

FastGene® restriction enzyme can cut substrate DNA in 5-15 min with FastGene® FastCut Buffer

1X FastGene® Buffer II

10 mM Tris-HCl (pH 7.9 at 25°C)
50 mM NaCl
10 mM MgCl₂
100 μ g/ml BSA

Unit definition

One unit is defined as the amount of enzyme required for complete digestion of 1 μ g bacteriophage λ at 37°C for 1 hr in 50 μ l reaction mixtures.

Quality control

- Unit definition assay
- Overdigestion assay
- Endonuclease assay
- Extreme pure assay

Standard reaction condition

- Normal protocol

| Component | Final Conc. | Volume |
|-------------------------|-------------|------------------|
| Substrate DNA | 1 μ g | X μ l |
| 10X FastGene® Buffer II | 1 X | 5 μ l |
| Sph I | 5 unit | 1 μ l |
| Sterile water | | up to 50 μ l |

→ Incubate at 37°C for 1 hr

- Fast protocol

| Component | Final Conc. | Volume |
|------------------------------|-------------|------------------|
| Substrate DNA | 1 μ g | X μ l |
| 10X FastGene® FastCut Buffer | 1 X | 5 μ l |
| Sph I | 5 unit | 1 μ l |
| Sterile water | | up to 50 μ l |

→ Incubate at 37°C for 15 min

※ We recommend 5-10 units of enzyme per μ g DNA and 10-20 units for genomic DNA in a 1 h digest.

Dilution buffer

FastGene® Diluent B

Heat Inactivation

Sph I can be inactivated at 65°C for 20 min.

Methylation sensitivity

dam methylation: Not sensitive*dcm* methylation: Not sensitive

CpG methylation: Not sensitive

Prolonged incubation

A minimum amount of enzyme required to digest 1 μ g substrate DNA for 16 hr; 0.13 U.

Relative activity in FastGene® Buffers

| | |
|---------------------------|------|
| FastGene® Buffer I: | 50% |
| FastGene® Buffer II: | 100% |
| FastGene® Buffer III: | 50% |
| FastGene® Buffer IV: | 75% |
| FastGene® FastCut Buffer: | 100% |

Note

It produces a 3' CATG extension, which can be efficiently ligated to DNA fragments cleaved by Nla III. It is not affected by *dam*, *dcm*, or mammalian CpG methylation. Phenol extraction is not suitable to isolate Sph I-cleaved DNA fragments due to a tight association of Sph I with DNA. Low concentration of NaCl enhances aggregation.