

SphI

Catalog: RK21160

Size: 500 U / 2,500 U

Concentration: 20,000 U/ml

Components:

SphI (20,000 U/ml)	RM21660
10X Buffer CutU	RM20102

Product Description

Restriction site:



Unit Definition:

One unit is defined as the amount of enzyme required to digest 1 µg of λ DNA fragments in 1 hour at 37°C in a total reaction volume of 50 µl.

Storage Conditions:

10 mM Tris-HCl, 100 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 200 µg/ml BSA, 50% Glycerol, pH 7.4 @ 25°C

Storage Temperature: -20°C

Reaction Conditions:

1X Buffer CutU. Incubate at 37°C.

1X Buffer CutU: 20 mM Tris-acetate, 10 mM Magnesium acetate, 50 mM Potassium acetate, 100 µg/ml BSA, pH 7.9 @25°C

Quick Cut: Yes

This enzyme will digest unit substrate in 5-15 minutes under recommended reaction conditions.

Heat Inactivation: 80°C for 20 min

Methylation Sensitivity:

dam Methylation	Not Sensitive
dcm Methylation	Not Sensitive
CpG Methylation	Not Sensitive

Instructions

A "Typical" SphI Digest:

Composition	Amount
ddH ₂ O	Up to 50 µL
10x Buffer CutU	5 µL
DNA*	1 µg
SphI**	0.5-1µL

*DNA substrates should be free of phenol, chloroform, ethanol, EDTA, detergents or high concentrations of salt, otherwise it will affect the enzyme activity.

** For complete digestion of 1 µg of plasmid DNA in 50 µL reaction, 0.5µL is sufficient, generally 1 µL is used.

QC Process:

- ◆ Purity is above 95% detected by SDS-PAGE.
- ◆ No exonuclease, nuclease contamination.
- ◆ No residual host genomic DNA is detected by PCR.

Optimizing Restriction Endonuclease Reactions

There are several key factors to consider when setting up a restriction endonuclease digest. Using the proper amounts of DNA, enzyme and buffer components in the correct reaction volume will allow you to achieve optimal digestion. By definition, 1 unit of restriction enzyme will completely digest 1 µg of substrate DNA in a 50 µl reaction in 60 minutes. This enzyme : DNA : reaction volume ratio can be used as a guide when designing reactions. However, most researchers follow the "typical" reaction conditions listed, where a 5–10 fold overdigestion is recommended to overcome variability in DNA source, quantity and purity.

A "Typical" Restriction Digest

Restriction Enzyme	0.5µL is sufficient, generally 1 µL is used
DNA	1 µg
10X ABclonal Buffer	5 µl (1X)
Total Reaction Volume	50 µL
Incubation Time	1 hour*
Incubation Temperature	Enzyme dependent

* Can be decreased to 5-15 minutes by using a "Quick Cut" Restriction Enzyme.

Enzyme

- Keep on ice when not in the freezer.
- Should be the last component added to reaction.
- Mix components by pipetting the reaction mixture up and down, or by "flicking" the reaction tube. Follow with a quick ("touch") spin-down in a microcentrifuge. Do not vortex the reaction.
- In general, we recommend 5–10 units of enzyme per µg DNA, and 10–20 units for genomic DNA in a 1 hour digest.

DNA

- Should be free of contaminants such as phenol, chloroform, alcohol, EDTA, detergents or excessive salts. Extra wash steps during purification are recommended.
- Methylation of DNA can inhibit digestion with certain enzymes.

Buffer

- Use at a 1X concentration
- Supplement with SAM (S-Adenosyl methionine) to the recommended concentration if required.

Reaction Volume

- A 50 µl reaction volume is recommended for digestion of 1 µg of substrate.
- Enzyme volume should not exceed 10% of the total reaction volume to prevent star activity due to excess glycerol.
- Additives in the restriction enzyme storage buffer (e.g., glycerol, salt) as well as contaminants found in the substrate solution (e.g., salt, EDTA, or alcohol) can be problematic in smaller reaction volumes. The following guidelines can be used for techniques that require smaller reaction volumes.

Reaction System	Enzyme Amount*	DNA	10X ABclonal Buffer
10 µl**	1 U	0.1 µg	1 µl
25 µl	5 U	0.5 µg	2.5 µl
50 µl	10 U	1 µg	5 µl

* Restriction Enzymes should be diluted when smaller amounts are needed.

** 10 µl rxns should not be incubated for longer than 1 hour to avoid evaporation.

Incubation Time

- Incubation time is typically 1 hour.
- Can often be decreased by using an excess of enzyme, or by using one of our "Quick Cut" restriction enzymes.
- It is possible, with many enzymes, to use fewer units and digest for up to 16 hours.

Stopping a Reaction

If no further manipulation of DNA is required:

- Terminate with a stop solution (10 µl per 50 µl rxn) [1x: 2.5% FicoII-400, 10 mM EDTA, 3.3 mM Tris-HCl, 0.08% SDS, 0.02% Tartrazine, 0.001% Xylene Cyanol FF, pH 8.0 @ 25°C].

When further manipulation of DNA is required:

- Heat inactivation can be used.
- Remove enzyme by using a spin column or phenol/chloroform extraction

Control Reactions

If you are having difficulty cleaving your DNA substrate, we recommend the following control reactions:

- Control DNA (DNA with multiple known sites for the enzyme, e.g. lambda or pUC19 DNA) with restriction enzyme to test enzyme viability.

If the control DNA is cleaved and the experimental DNA resists cleavage, the two DNAs can be mixed to determine if an inhibitor is present in the experimental sample. If an inhibitor (often salt, EDTA or phenol) is present, the control DNA will not cut after mixing.

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