SfiI

Catalog: RK21105
Size: 3,000 U / 15,000 U
Concentration: 20,000 U/ml
Components:

SfiI (20,000 U/ml)	RM21611
10X Buffer CutS	RM20103

Product Description:

Restriction site:

Unit Definition:

One unit is defined as the amount of enzyme required to digest 1 μg of pXba DNA in 1 hour at 50 ℃ in a total reaction volume of 50 μl.

Storage Conditions:

10 mM Tris-HCl, 250 mM NaCl, 0.1 mM EDTA, 200 μg/ml BSA, 50% Glycerol, 0.15% Triton® X-100, 1 mM DTT, pH 7.4 @ 25 ℃

Storage Temperature: -20℃

Reaction Conditions:

1X Buffer CutS, Incubate at 50 ℃.

1X Buffer CutS: 50 mM Potassium Acetate, 20 mM Tris-acetate, 10 mM Magnesium Acetate, 100 μg/ml BSA, pH 7.9 @ 25 ℃

Quick Cut: Yes

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

Activity in ABclonal Buffer

CutA	CutB	CutC	CutS
25%	100%	50%	100%

Heat Inactivation: No

Methylation Sensitivity:

dam methylation	Not Sensitive
dcm methylation	Impaired by Overlapping
CpG Methylation	Blocked by Some Combinations of Overlapping

Activity at Temperature:

@37 ℃: 10%

Instructions:

A "Typical" SfiI Digest:

Composition	Amount
H_2O	Up to 50 μl
10x Buffer CutS	5 μl
DNA*	1 μg
SfiI	0.5-1μl 10 units is sufficient, generally 1 μl is used

- The substrates are completely digested in 5-15 min incubate at 50 ℃.
- *DNA substrates should be free of phenol, chloroform, ethanol, EDTA, detergents or high concentrations of salt, otherwise it will affect the enzyme activity.
- For enzymes that cannot be heat-inactivated, we recommend using a column for cleanup, or running the reaction on an agarose gel and then extracting the DNA, or performing a phenol/chloroform extraction.
- SfiI requires two copies of its recognition sequence for cleavage to occur. The two sites can be on either the same or different DNA molecules. Wertzell L.M. et al., (1995) J. Mol. Biol. 248:581-595.

QC Process:

- Purity is above 95% detected by SDS-PAGE.
- No exonuclease, nuclease contamination.
- Host genomic DNA is no residual 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

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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	10 units 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% Ficoll-400, 10 mM EDTA, 3.3 mM Tris-HCl, 0.08% SDS, 0.02% Tartrazine, 0.001% Xylene Cyanol FF, pH 8.0 @ 25℃].

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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