

## SacI

**Product Number: SA02**

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### Shipping and Storage

Stored at -20°C, valid for two years.

### Components

|                         |       |
|-------------------------|-------|
| Component               | SA02  |
|                         | 100µl |
| SacI                    | 100µl |
| 10×Cut Buffer           | 1ml   |
| Easy-Load 10×Cut Buffer | 1ml   |

### Description

SacI is a high-quality restriction endonuclease that has been genetically engineered and can quickly complete DNA cleavage using only one buffer within 5-15 minutes. Suitable for rapid enzymatic digestion of plasmid DNA, PCR products, or genomic DNA.

- Enzyme activity detection:** At the optimal reaction temperature, in a 20µl reaction system, 1µl SacI can completely digest 1µg of λ DNA (HindIII digest) within 15 minutes.
- Long term enzyme digestion detection:** Incubate 1µl SacI with 1µg λ DNA (HindIII digest) for 3 hours at the optimal reaction temperature, and no non-specific degradation of the substrate caused by other nucleases contamination or star activity was detected. Delayed enzyme digestion may result in star activity.
- Enzyme digestion ligation re digestion detection:** At the optimal reaction temperature, use 1µl SacI to digest the substrate, recover the enzyme digestion product, and use an appropriate amount of T4 DNA Ligase at 22 °C to reconnect the enzyme digestion product. After recovering the ligation product again, use the same endonuclease to cleave the ligation product again.
- Detection of non-specific endonuclease activity:** at the optimal reaction temperature, 1µl SacI and 1µg super spiral plasmid DNA were incubated together for 4h, and agarose gel electrophoresis was used to detect that the plasmid DNA was still in the super spiral state.
- Blue white spot detection:** The vector containing a single lacZα gene was digested with 1µl SacI, reconnected, and transformed into competent E. coli cells. The cells were then coated on LB medium plates containing corresponding antibiotics, IPTG, and X-gal. Products with correct connections will grow blue colonies, while products with incorrect connections (i.e. incomplete DNA end incisions) will grow white colonies. For our restriction enzyme, the proportion of white colonies should be less than 1%

### Basic information

| Recognition sequence                                     | Isoschizomer      | Enzyme digestion temperature | Deactivation conditions | Methylation interference? |
|--|-------------------|------------------------------|-------------------------|---------------------------|
| 5'-GAGCT <sup>^</sup> C-3'<br>3'-C <sup>^</sup> TCGAG-5' | Psp124BI,<br>SstI | 37°C                         | 80°C 20min              | No effect                 |

**The activity (buffer compatibility) in different reaction buffers is as follows:**

| 10×Cut Buffer | Easy-Load 10×Cut Buffer | Thermo FastDigest Buffer | NEB CutSmart® Buffer | Takara QuickCut™ Buffer |
|---------------|-------------------------|--------------------------|----------------------|-------------------------|
| 100%          | 100%                    | 100%                     | 100%                 | 100%                    |

**Please refer to the table below for the methylation effects of SacI recognition sites:**

| Dam       | Dcm       | CpG       | EcoKI     | EcoBI     |
|-----------|-----------|-----------|-----------|-----------|
| No effect | No effect | No effect | No effect | No effect |



### Features

1. Enzymatic cleavage can be completed within 5-15 minutes;
2. All endonucleases share a single enzyme digestion buffer, Cut Buffer, greatly simplifying the enzyme digestion reaction system and facilitating double or multiple enzyme digestion;
3. In response to the issue of differences in activity of different enzymes in Cut Buffer, the concentrations of different enzymes were adjusted to uniformly add 1µl of enzyme per 20µl of system for enzyme digestion reaction;
4. Many modifying enzymes, such as Alkaline Phosphatase, Antarctic Phosphatase, T4 DNA Ligase, T4 Polynucleotide Kinase, T4 PNK (3' phosphatase minus), etc., are 100% compatible with Cut Buffer, making reaction systems such as "enzyme cut connect" and "enzyme cut modify connect" compatible and supporting single tube reactions;
5. Good enzyme activity redundancy makes it easy to cope with substrate excess or difficult template enzyme digestion.

### Note

1. Endonucleases should be stored in an ice box or on an ice bath during use, and should be immediately stored at -20°C after use.
2. If it is found that the expected enzyme cleavage site cannot be cleaved, please confirm whether there is methylation interference.
3. Homolytic enzymes may have different sensitivities to different methylation modifications, and when encountering potential methylation interference issues, they can be attempted.
4. This product is only for scientific research by professionals and should not be used for clinical diagnosis or treatment, food or medicine, or stored in ordinary residential areas.
5. For your safety and health, please wear lab coats and disposable gloves when operating.

### Protocol

1. When performing single enzyme digestion, the following reaction system can be referred to and operated on an ice bath.

| Reagent                                  | Plasmid DNA    | PCR Product | Genomic DNA |
|--|----------------|-------------|-------------|
| Ultrapure Water                          | (17-x)µl       | (26-x)µl    | (40-x)µl    |
| 10×Cut Buffer or Easy-Load 10×Cut Buffer | 2µl            | 3µl         | 5µl         |
| Substrate DNA                            | xµl(up to 1µg) | xµl(~0.2µg) | xµl(5µg)    |
| SacI                                     | 1µl            | 1µl         | 5µl         |
| Total volume                             | 20µl           | 30µl        | 50µl        |
| Incubate at 37°C                         | 15min          | 15-30min    | 30-60min    |

**Note: The above reaction system is applicable for enzyme digestion of purified PCR products. The unpurified PCR product has a certain ionic strength and pH, and the amount of 10×Cut Buffer added can be appropriately reduced to 2µl. However, due to the simultaneous exonuclease activity of many DNA polymerases, it can affect the cleavage products. Therefore, the following steps require ligation, cloning, and other operations. It is recommended to purify the PCR products before cleavage.**

- 1.1. After adding various liquids in sequence according to the table above, use a pipette to gently suck or tap the tube wall to mix (do not vortex), and then centrifuge instantly to settle the liquid to the bottom of the tube.
- 1.2. Incubate at 37°C for 15 minutes (plasmid), 15-30 minutes (PCR product), or 30-60 minutes (genomic DNA). It is recommended to use a water bath as the preferred method for enzyme digestion reactions, as the reaction temperature is usually more constant.
- 1.3. Incubate at 80°C for 20 minutes to inactivate the enzyme and stop the reaction (optional).
2. When performing double or multiple enzyme digestion, the reaction system can be set according to the following principles based on the enzyme digestion reaction system settings in the reference form.
  - 2.1. The dosage of each rapid endonuclease is 1µl, and the reaction system should be appropriately expanded as needed.
  - 2.2. The total volume of all rapid endonucleases must not exceed 1/10 of the total reaction system.
  - 2.3. If the optimal reaction temperatures for the several rapid endonucleases used are different, the enzyme with the lower



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optimal temperature should be used first for digestion, and then the enzyme with the higher optimal temperature should be added for digestion reaction at its optimal reaction temperature.

### Related products

| Product Number | Product Name |
|----------------|--------------|
| APA01          | ApaLI        |
| AS01           | AscI         |
| AV01           | AvrII        |
| BAM01          | BamHI        |
| BC01           | BclI         |
| BG01           | BglII        |
| BSA01          | BsaI         |
| BSN01          | BspQI        |
| BSN02          | BstBI        |
| BSN03          | BstEII       |
| CF01           | Cfr9I        |
| CL01           | ClaI         |
| DPN01          | DpnI         |
| DPN02          | DpnII        |
| DRA01          | DraI         |
| EA01           | EagI         |
| EA02           | EarI         |
| EC02           | EcoRI        |
| EC01           | EcoRV        |
| FSP01          | FspI         |
| HID01          | HindIII      |
| HIN01          | Hinfi        |
| HPA01          | HpaI         |
| KAS01          | KasI         |
| KPN01          | KpnI         |
| ML01           | MluI         |
| RS02           | MnlI         |
| MSP01          | MspI         |
| NC01           | NcoI         |
| ND01           | NdeI         |
| NH01           | NheI         |
| NO01           | NotI         |
| NR01           | NruI         |
| NS01           | NsiI         |
| PA01           | PacI         |
| PM01           | PmeI         |
| PS01           | PstI         |
| PV01           | PvuII        |
| SA02           | SacI         |
| SA05           | SacII        |

**For Research Use Only**



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|       |      |
|-------|------|
| SA03  | SaI  |
| SA04  | SapI |
| SB02  | SbfI |
| SC02  | ScaI |
| SF01  | SfiI |
| SM01  | SmaI |
| SP01  | SpeI |
| SP02  | SphI |
| SSP01 | SspI |
| STE01 | StuI |
| TAQ01 | TaqI |
| XB01  | XbaI |
| XC01  | XcmI |
| XH01  | XhoI |
| XM01  | XmaI |