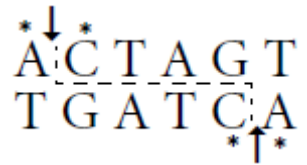


For life science research only.
Not for use in diagnostic procedures.



Restriction Endonuclease Spe I from *Sphaerotilus species*



Version: 16

Content Version: November 2020

Cat. No. 11 008 951 001 1,000 U
 10 U/μl

Store product at –15 to –25°C.

| | | |
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1. General Information

1.1. Contents

| Vial / Bottle | Cap | Label | Function / Description | Content |
|---------------|-----|--|---|------------------------------|
| Spe I | red | Spe I | Contains 20 mM Tris-HCl, 100 mM NaCl, 0.1 mM EDTA, 10 mM 2-mercaptoethanol, 0.2% Triton X-100 (v/v), 50% glycerol (v/v), pH approximately 8.0 (+4°C). | 1 vial, 1,000 U (10 U/μl) |
| H | red | SuRE/Cut Buffer H for Restriction Enzymes, 10x conc. | Contains 500 mM Tris-HCl, 1 M NaCl, 100 mM MgCl ₂ , 10 mM dithioerythritol, pH 7.5 (+37°C). | 1 vial, 1 ml |

1.2. Storage and Stability

Storage Conditions (Product)

The product is shipped on dry ice.

When stored at –15 to –25°C, the product is stable through the expiration date printed on the label.

| Vial / Bottle | Cap | Label | Storage |
|---------------|-----|------------------------------|--|
| Spe I | red | Spe I | Store at –15 to –25°C. ⚠ Do not store below –25°C. |
| H | red | SuRE/Cut Buffer H, 10x conc. | Store at –15 to –25°C. |

1.3. Application

Spe I recognizes the sequence A/CTAGT and generates fragments with 5'-cohesive termini (Roberts RJ, 1985).

2. How to Use this Product

2.1. Before you Begin

Safety Information

For customers in the European Economic Area

Contains SVHC: octyl/nonylphenol ethoxylates. For use in research and under controlled conditions only – acc. to Art. 56.3 and 3.23 REACH Regulation.

2.2. Protocols

The following steps describe a typical experiment.

- 1 Prepare the restriction digest according to the following table.

| Reagent | Final conc. |
|-----------------------|-----------------------------|
| DNA | 1 µg |
| 10x SuRE/Cut Buffer H | 2.5 µl |
| Water, PCR Grade* | Up to total volume of 25 µl |
| Spe I | 1 U |

- 2 Incubate at +37°C for 1 hour.

2.3. Parameters

Activity in PCR Buffer

Not tested.

Buffers

Activity in SuRE/Cut Buffer System

| A | H ⁽¹⁾ | M |
|------------|---------------------|------|
| 75 to 100% | 100% ⁽²⁾ | 100% |

⁽¹⁾ Supplied Buffer

⁽²⁾ Indicates recommended buffer for optimal activity.

Cleavage Sites

Number of cleavage sites on different DNAs

| λ | Ad2 | SV40 | ΦX174 | M13mp7 | pBR322 | pBR328 | pUC18 |
|---|-----|------|-------|--------|--------|--------|-------|
| 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 |

Compatible Ends

Spe I generates compatible ends to Bln I, Nhe I, and Xba I.

| Enzyme with compatible ends | Recognition sequence | New sequence if Spe I is ligated to enzyme with compatible ends | | Enzyme that can cut this new sequence |
|-----------------------------|----------------------|---|----------------|---------------------------------------|
| | | Spe I – Enzyme | Enzyme – Spe I | |
| Bln I | C/CTAGG | A/CTAGG | C/CTAGT | Mae I |
| Nhe I | G/CTAGC | A/CTAGC | G/CTAGT | Mae I |
| Spe I | A/CTAGT | A/CTAGT | A/CTAGT | Spe I |
| Xba I | T/CTAGA | A/CTAGA | T/CTAGT | Mae I |

Inactivation

Spe I can be heat inactivated by incubation at +65°C for 15 minutes (tested up to 100 U/μg DNA).

Isoschizomers

Spe I is an isoschizomer to Bcu I and Ahi I.

Methylation Sensitivity

Spe I is inhibited by the presence of N⁶-methyladenine and 5'-methylcytosine in the sequence of ^mA/^mCTAGT, as indicated*.

Recognition Sites

*A*CTAGT

i * indicates methylation sensitivity.

Temperature Optimum

+37°C

Unit Definition

One unit is the enzyme activity that completely cleaves 1 μg pTRE57 DNA in one hour at +37°C in a total volume of 25 μl SuRE/Cut Buffer H.

3. Troubleshooting

| Observation | Possible cause | Recommendation |
|--|---|---|
| Inhibition or alteration of recognition specificity of restriction enzyme. | Compounds were used in the isolation of the DNA substrate, such as phenol, chloroform, ethanol, SDS, high levels of NaCl, and metal ions, such as Hg ²⁺ and Mn ²⁺ . | Remove compounds by ethanol precipitation followed by drying, before adding DNA to the restriction digest reaction. <hr/> Mix vial of restriction enzyme gently but completely prior to use. |

4. Additional Information on this Product

4.1. Test Principle

Commonly used bacterial strains

| Strain | Genotype |
|-----------------------|---|
| BL21 | <i>E. coli</i> B F ⁻ <i>dcm ompT hsdS</i> (r _B ⁻ m _B ⁻) <i>gal</i> (Studier FW, et al, 1986). |
| C600 ^e | <i>supE44 hsd R2 thi-1 thr-1 leuB6 lacY1 tonA21</i> (Hanahan D, 1983). |
| DH5α | <i>supE44 Δ(lacU169 (Φ80dlacZΔM15) hsdR17 recA1 endA1 gyrA96 thi-1 relA1</i> (Hanahan D, 1983). |
| HB101 | <i>supE44 hsdS20 recA13 ara-14 proA2 lacY1 galk2 rpsL20 xyl-5 mtl-1</i> (Hanahan D, 1983). |
| JM108 | <i>recA1 supE44 endA1 hsdR17 gyrA96 relA1 thi Δ(lac-proAB)</i> (Yanisch-Perron C, et al, 1985). |
| JM109 | <i>recA1 supE44 endA1 hsdR17 gyrA96 relA1 thi Δ(lac-proAB) F'[traD36proAB⁺, lacI^q lacZΔM15]</i> (Yanisch-Perron C, et al, 1985). |
| JM110 | <i>rpsL (Str^r) thr leu thi-I lacY galk galT ara tonA tsx dam dcm supE44 Δ(lac-proAB) F'[traD36proAB⁺, lacI^q lacZΔM15]</i> (Yanisch-Perron C, et al, 1985). |
| K802 | <i>supE hsdR gal metB</i> (Raleigh E, et al, 1986; Wood WB, 1966). |
| SURE ^r | <i>recB recJ sbc C201 uvrC umuC::Tn5(kan^r) lac, Δ(hsdRMS) endA1 gyrA96 thi relA1 supE44 F'[proAB⁺ lacI^q lacZΔM15 Tn10 (tet^r)</i> (Greener A, 1990). |
| TG1 | <i>supE hsd Δ5 thi Δ(lac-proAB) F'[traD36proAB⁺, lacI^q lacZΔM15]</i> (Gibson TJ, 1984). |
| XL1-Blue ^r | <i>supE44 hsdR17 recA1 endA1 gyrA46 thi relA1 lac F'[proAB⁺, lacI^q lacZΔM15 Tn10 (tet^r)</i> (Bullock WO, et al, 1987). |

4.2. References

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- Raleigh EA, Wilson G. Escherichia coli K-12 restricts DNA containing 5-methylcytosine. *Proc Natl Acad Sci USA.*1986;83:9070-9074.
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- Yanisch-Perron C, Vieira J, Messing J. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. *Gene.*1985;33:103-19.

4.3. Quality Control

For lot-specific certificates of analysis, see section **Contact and Support**.

5. Supplementary Information

5.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols

 **Information Note:** Additional information about the current topic or procedure.

 **Important Note:** Information critical to the success of the current procedure or use of the product.

① ② ③ etc. Stages in a process that usually occur in the order listed.

① ② ③ etc. Steps in a procedure that must be performed in the order listed.

* (Asterisk) The Asterisk denotes a product available from Roche Diagnostics.

5.2. Changes to previous version

Layout changes.

Editorial changes.

New information added related to the REACH Annex XIV.

5.3. Ordering Information

| Product | Pack Size | Cat. No. |
|--------------------|-----------------------------|----------------|
| Reagents, kits | | |
| T4 DNA Ligase | 100 U, 1 U/μl | 10 481 220 001 |
| | 500 U, 1 U/μl | 10 716 359 001 |
| | 500 U, 5 U/μl | 10 799 009 001 |
| Water, PCR Grade | 25 ml, 25 x 1 ml | 03 315 932 001 |
| | 25 ml, 1 x 25 ml | 03 315 959 001 |
| | 100 ml, 4 x 25 ml | 03 315 843 001 |
| SuRE/Cut Buffers | SuRE/Cut Buffer A, 5 x 1 ml | 11 417 959 001 |
| | SuRE/Cut Buffer M, 5 x 1 ml | 11 417 983 001 |
| | SuRE/Cut Buffer H, 5 x 1 ml | 11 417 991 001 |
| 1,4-Dithiothreitol | 2 g | 10 197 777 001 |
| | 10 g | 10 708 984 001 |
| | 25 g | 11 583 786 001 |

5. Supplementary Information

5.4. Trademarks

All product names and trademarks are the property of their respective owners.

5.5. License Disclaimer

For patent license limitations for individual products please refer to:

List of biochemical reagent products.

5.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

5.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

5.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site.**

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

