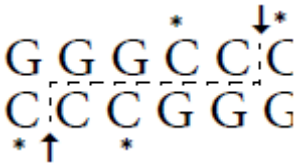
For life science research only.
Not for use in diagnostic procedures.

Restriction Endonuclease Apa I

from *Acetobacter pasteurianus*



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Cat. No. 10 703 753 001 20,000 U
40 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
Apa I conc.	purple	Apa I, high conc.	Contains 20 mM Tris-HCl, 100 mM NaCl, 0.1 mM EDTA, 10 mM 2-mercaptoethanol, 0.02% polydocanol (v/v), 50% glycerol (v/v), pH approximately 8.0 (+4°C).	1 vial, 20,000 U (40 U/µl)
A	purple	SuRE/Cut Buffer A for Restriction Enzymes, 10x conc.	Contains 330 mM Tris acetate, 660 mM potassium acetate, 100 mM magnesium acetate, 5 mM dithiothreitol, pH 7.9 (+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
Apa I conc.	purple	Apa I, high conc.	Store at −15 to −25°C. ⚠ ***Do not store below −25°C.***
A	purple	SuRE/Cut Buffer A, 10x conc.	Store at −15 to −25°C.

1.3. Application

Apa I recognizes the sequence GGGCC/C and generates fragments with 3′-cohesive termini (Seurinck J, Van de Voorde A, Van Montagu M, 1983).

2. How to Use this Product

2.1. 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 A	2.5 µl
Water, PCR Grade*	Up to total volume of 25 µl
Apa I	1 U

2. Incubate at +30°C for 1 hour.

2.2. Parameters

Activity in PCR Buffer

100%
Relative activity in PCR mix (Taq DNA Polymerase buffer) is 100%. The PCR mix contained λ target DNA, primers, 10 mM Tris-HCl (pH 8.3, +20°C), 50 mM KCl, 1.5 mM $MgCl_2$, 200 µM dNTPs, 2.5 U Taq DNA polymerase. The mix was subjected to 25 amplification cycles.

Buffers

Activity in SuRE/Cut Buffer System

A[1]	H	M
100%[2]	0 to 10%	50 to 75%

[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
1	12	1	0	0	0	0	0

Compatible Ends

Apa I has no compatible ends to other known restriction enzymes.

Inactivation

Apa I can be heat inactivated by incubation at +65°C for 15 minutes.

Isoschizomers

Apa I is an isoschizomer to Bsp 120 I and PspOMI.

Methylation Sensitivity

Apa I is inhibited by the presence of 5′-methylcytosine.

Recognition Sites

GGG*CC*C

** indicates methylation sensitivity.*

Temperature Optimum

+30°C

Apa I has a special incubation temperature.

Unit Definition

One unit is the enzyme activity that completely cleaves 1 µg Hind III digested λDNA in one hour at +30°C in a total volume of 25 µl SuRE/Cut Buffer A.

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.
		Mix vial of restriction enzyme gently but completely prior to use.

4. Additional Information on this Product

4.1. Test Principle

Absence of nonspecific endonuclease activities

1 µg Hind III digested λDNA is incubated for 16 hours in 50 µl SuRE/Cut Buffer A with excess of Apa I. The number of enzyme units which do not change the enzyme-specific pattern is stated in the certificate of analysis.

Absence of exonuclease activity

Approximately 5 µg [^{3}H]-labeled calf thymus DNA are incubated with 3 µl Apa I for 4 hours at +37°C in a total volume of 100 µl 50 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM dithioerythritol, pH approximately 7.5. Under these conditions, no release of radioactivity is detectable as stated in the certificate of analysis.

Ligation and recutting assay

Apa I fragments obtained by full digestion of 1 µg Hind III digested λDNA are ligated with 1 U T4 DNA Ligase* in a volume of 10 µl by incubation for 16 hours at +4°C in 66 mM Tris-HCl, 5 mM $MgCl_2$, 5 mM Dithiothreitol*, 1 mM ATP, pH 7.5 (+20°C) resulting in >95% recovery of 1 µg Hind III digested λDNA fragments. Subsequent recutting with Apa I yields >95% of the typical pattern of 1 µg Hind III and Apa I digested λDNA.

Commonly used bacterial strains

Strain	Genotype
BL21	*E. coli B F$^-$ dcm ompT hsdS(r_B- m_B-) gal* (Studier FW, et al, 1986).
C600^e	*supE44 hsd R2 thi-1 thr-1 leuB6 lacY1 tonA21* (Hanahan D, 1983).
DH5α	*supE44* Δ(*lac*U169 (Φ80d*lac*ZΔM15) *hsd*R17 *rec*A1 *end*A1 *gyr*A96 *thi*-1 *rel*A1 (Hanahan D, 1983).
HB101	*supE44 hsdS20 recA13 ara-14 proA2 lacY1 galK2 rpsL20 xyl-5 mtl-1* (Hanahan D, 1983).
JM108	*recA1 supE44 endA1 hsdR17 gyrA96 relA1 thi* Δ(*lac-pro*AB) (Yanisch-Perron C, et al, 1985).
JM109	*recA1 supE44 endA1 hsdR17 gyrA96 relA1 thi* Δ(*lac-pro*AB) F′[*traD36proAB*$^+$, *lac*I^q *lac*ZΔM15] (Yanisch-Perron C, et al, 1985).
JM110	*rpsL (Str^r) thr leu thi-l lacY galK galT ara tonA tsx dam dcm supE44 Δ(lac-proAB) F′[traD36proAB$^+$, lacI^q lacZΔM15]* (Yanisch-Perron C, et al, 1985).
K802	*supE hsdR gal metB* (Raleigh E, et al, 1986; Wood WB, 1966).
SURE^r	*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) (Greener A, 1990).
TG1	*supE hsd Δ5 thi* Δ(*lac-pro*AB) F′[*traD36proAB*$^+$, *lac*I^q *lac*ZΔM15] (Gibson TJ, 1984).
XL1-Blue^r	*supE44 hsdR17 recA1 endA1 gyrA46 thi relA1 lac* F′[*proAB*$^+$, *lac*I^q *lac*ZΔM15 Tn10 *(tet^r)*] (Bullock WO, et al, 1987).

4.2. References

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- Hanahan D. Studies on transformation of Escherichia coli with plasmids. J Mol Biol.1983;166:557-580.
- 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.
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- Bullock WO, Fernandez JM, Short JM. XL1-Blue– a high-efficiency plasmid transforming recA Escherichia coli strain with β-galactosidase selection. BioTechniques. 1987;5:376-379.
- Seurinck J, Van de Voorde A, Van Montagu M. A new restriction endonuclease from Acetobacter pasteurianus. Nucleic Acids Res. 1983;11:4409-4415.

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.
❶ ❷ ❸ 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.

5.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
1,4-Dithiothreitol	2 g	10 197 777 001
	10 g	10 708 984 001
	25 g	11 583 786 001
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 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

5.4. Trademarks

SURE/CUT is a trademark of Roche.
All other 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.

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