

Restriction Endonuclease Dde I

From *Desulfovibrio desulfuricans*, strain Norway

Cat. No. 10 835 307 001 1000 units (10 U/μl)



Version 18

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Store at -15 to -25°C

Stability/Storage When stored at -15 to -25°C the undiluted enzyme solution is stable until the expiration date printed on the label. Do not store below -25°C to avoid freezing.

Sequence specificity *Dde* I recognizes the sequence C/TNAG and generates fragments with 5'-cohesive termini (1).

Compatible ends *Dde* I generates compatible ends to *Aoc* I (*Sau* I), *Cel* II, *Mst* II and *Oxa* NI. However, ligation of these fragments is difficult due to the presence of an undefined nucleotide in the 5'-cohesive ends.

Enzyme with compatible ends	Recognition sequence	New sequence if <i>Dde</i> I is ligated to enzyme with compatible ends		Enzyme that can cut this new sequence
		<i>Dde</i> I - Enzyme	Enzyme - <i>Dde</i> I	
<i>Aoc</i> I	CC/TNAGG	C/TNAGG	CC/TNAG	<i>Dde</i> I
<i>Cel</i> II	GC/TNAGC	C/TNAGC	GC/TNAG	<i>Dde</i> I
<i>Dde</i> I	C/TNAG	C/TNAG	C/TNAG	<i>Dde</i> I
<i>Mst</i> II / <i>Oxa</i> NI	CC/TNAGG	C/TNAGG	CC/TNAG	<i>Dde</i> I

Isoschizomers The enzyme is not known to have isoschizomers.

Methylation sensitivity *Dde* I is inhibited by the presence of 5'-methyl- and 5'-hydroxymethylcytosine in the sequence *C/TNAG.

Storage buffer 10 mM Tris-HCl, 50 mM NaCl, 0.1 mM EDTA, 1 mM dithiothreitol, 0.01% Triton X-100, 50% glycerol (v/v), 500 μg bovine serum albumin, pH approx. 8.0 (at 4°C).

Supp. Incubation buffer (10x) 500 mM Tris-HCl, 1 M NaCl, 100 mM MgCl₂, 10 mM Dithioerythritol, pH 7.5 (at 37°C), (Δ SuRE/Cut Buffer **H**).

Activity in SuRE/Cut Buffer System

Bold face printed buffer indicates the recommended buffer for optimal activity:

A	B	L	M	H
50-75%	75-100%	25-50%	25-50%	100%

Incubation temperature **37°C**

Unit definition One unit is the enzyme activity that completely cleaves 1 μg λDNA in 1 h at **37°C** in a total volume of 25 μl SuRE/Cut buffer **H**.

Typical experiment

Component	Final concentration
DNA	1 μg
10 × SuRE/Cut Buffer H	2.5 μl
Sterile redist. water	Up to a total volume of 25 μl
Restriction enzyme	1 unit

Incubate at **37°C** for 1 h.

Heat Inactivation *Dde* I is not heat-inactivated by 15 min incubation at 65°C.

Number of cleavage sites on different DNAs (2):

λ	Ad2	SV40	Φ X174	M13mp7	pBR322	pBR328	pUC18
104	97	20	14	29	8	9	6

Activity in PCR buffer

Relative activity in PCR mix (Taq DNA Polymerase buffer) is **40%**. The PCR mix contained λ target DNA, primers, 10 mM Tris-HCl (pH 8.3, 20°C), 50 mM KCl, 1.5 mM MgCl₂, 200 μM dNTPs, 2.5 U Taq DNA polymerase. The mix was subjected to 25 amplification cycles.

Troubleshooting

A critical component is the DNA substrate. Many compounds used in the isolation of DNA such as phenol, chloroform, ethanol, SDS, high levels of NaCl, metal ions (e.g., Hg²⁺, Mn²⁺) inhibit or alter recognition specificity of many restriction enzymes. Such compounds should be removed by ethanol precipitation followed by drying, before the DNA is added to the restriction digest reaction. Appropriate mixing of the enzyme is recommended.

Star Activity

The enzyme has been shown to exhibit relaxed specificity when used in large excess.

Quality control

Lot-specific certificates of analysis are available at www.lifescience.roche.com/certificates.

Absence of unspecific endonuclease activities

1 μg λDNA is incubated for 16 h in 50 μl SuRE/Cut buffer H with excess of *Dde* 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

Approx. 5 μg [³H] labeled calf thymus DNA are incubated with 3 μl *Dde* I for 4 h at 37°C in a total volume of 100 μl 50 mM Tris-HCl, 10 mM MgCl₂, 1 mM dithioerythritol, pH approx. 7.5. The release of radioactivity is calculated as a percentage value of liberated to input radioactivity per unit of enzyme (stated in the certificate of analysis).

Ligation and recutting assay

Dde I fragments obtained by complete digestion of 1 μg pBR322 × DNA are ligated with 1 U T4 DNA Ligase (Cat. No. 10 481 220 001) in a volume of 10 μl by incubation for 16 h at 4°C in 66 mM Tris-HCl, 5 mM MgCl₂, 5 mM Dithiothreitol, 1 mM ATP, pH 7.5 (at 20°C).

The percentage of ligation and subsequent recutting with *Dde* I which yields the typical pattern of pBR322 × *Dde* I fragments is determined and stated in the certificate of analysis.

References

- Makula, R. A. & Maegher, R. B. (1980) *Nucleic Acids Res.* **8**, 3125-3131.
- Kessler, C. & Manta, V. (1990) *Gene* **92**, 1-248.
- Rebase The Restriction Enzyme Database: <http://rebase.neb.com>
- Vale, A. et al (2003) *Int. J. Med. Microbiol.* **293**, 109.

Ordering Information

Product	Application	Packsizes	Cat. No.
Restriction Enzymes	DNA restriction digestion	Please refer to website	
T4 DNA Ligase	Ligation of sticky- and blunt-ended DNA fragments.	100 U 500 units (1 U/μl)	10 481 220 001 10 716 359 001
SuRE/Cut Buffer Set for Restriction Enzymes	Incubation buffers A, B, L, M and H for restriction enzymes	1 ml each (10× conc. solutions)	11 082 035 001
SuRE/Cut Buffer A	Restriction enzyme incubation	5 × 1 ml (10× conc. solution)	11 417 959 001
SuRE/Cut Buffer B	Restriction enzyme incubation	5 × 1 ml (10× conc. solution)	11 417 967 001
SuRE/Cut Buffer H	Restriction enzyme incubation	5 × 1 ml (10× conc. solution)	11 417 991 001
SuRE/Cut Buffer L	Restriction enzyme incubation	5 × 1 ml (10× conc. solution)	11 417 975 001
SuRE/Cut Buffer M	Restriction enzyme incubation	5 × 1 ml (10× conc. solution)	11 417 983 001
Water, PCR Grade	Specially purified, double-distilled, deionized, and autoclaved	100 ml (4 vials of 25 ml)	03 315 843 001
		25 ml (25 vials of 1 ml)	03 315 932 001
		25 ml (1 vial of 25 ml)	03 315 959 001

Changes to previous version

Editorial changes.

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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, F.W. <i>et al</i> (1986) <i>J. Mol. Biol.</i> , 189 , 113.)
C600 ^e	<i>supE44 hsdR2 thi-1 thr-1 leuB6 lacY1 tonA21</i> ; (Hanahan, D. (1983) <i>J. Mol. Biol.</i> 166 , 557.)
DH5α	<i>supE44 Δ(lacU)169 (φ80d/lacZΔM15) hsdR17 recA1 endA1 gyrA96 thi-1 relA1</i> ; (Hanahan, D. (1983) <i>J. Mol. Biol.</i> 166 , 557.)
HB101	<i>supE44 hsdS20 recA13 ara-14 proA2 lacY1 galK2 rpsL20 xyl-5 mtl-1</i> ; (Hanahan, D., (1983) <i>J. Mol. Biol.</i> 166 , 557.)
JM108	<i>recA1 supE44 endA1 hsdR17 gyrA96 relA1 thi Δ(lac-proAB)</i> ; (Yanisch-Perron, C. <i>et al.</i> , (1985) <i>Gene</i> 33 , 103.)
JM109	<i>recA1 supE44 endA1 hsdR17 gyrA96 relA1 thi Δ(lac-proAB) F[traD36proAB⁺, lac^q lacZΔM15]</i> ; (Yanisch-Perron, C. <i>et al.</i> , (1985) <i>Gene</i> 33 , 103.)
JM110	<i>rpsL (Str^r) thr leu thi-1 lacY galK galT ara tonA tsx dam dcm supE44 Δ(lac-proAB) F[traD36proAB⁺, lac^q lacZΔM15]</i> ; (Yanisch-Perron, C. <i>et al.</i> , (1985) <i>Gene</i> 33 , 103.)
K802	<i>supE hsdR gal metB</i> ; (Raleigh, E. <i>et al.</i> , (1986) <i>Proc.Natl. Acad.Sci USA</i> , 83 , 9070.; Wood, W.B. (1966) <i>J. Mol. Biol.</i> , 16 , 118.)
SURE ^f	<i>recB recJ sbc C201 uvrC umuC::Tn5(kan^r) lac</i> , Δ(<i>hsdRMS</i>) <i>endA1 gyrA96 thi relA1 supE44 F[proAB⁺ lac^q lacZΔM15 Tn10 (tet^r)</i> ; (Greener, A. (1990) <i>Stratagies</i> , 3 , 5.)
TG1	<i>supE hsd Δ5 thi Δ(lac-proAB) F[traD36proAB⁺, lac^q lacZΔM15]</i> ; (Gibson, T.J. (1984) <i>PhD Theses. Cambridge University, U.K.</i>)
XL1-Blue ^f	<i>supE44 hsdR17 recA1 endA1 gyrA46 thi relA1 lac F[proAB⁺, lac^q lacZΔM15 Tn10 (tet^r)</i> ; (Bullock <i>et al.</i> , (1987) <i>BioTechniques</i> , 5 , 376.)

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