

Restriction Endonuclease Dpn I

From *Diplococcus pneumoniae*

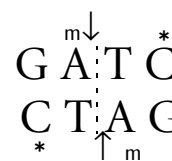
Cat. No. 10 742 970 001

200 units (10 U/ μ l)

Cat. No. 10 742 988 001

1000 units (10 U/ μ l)

Please see label for lot specific values.



Version June 2005

Store at -15 to -25°C

Stability/Storage

The undiluted enzyme solution is stable when stored at -15 to -25°C until the control date printed on the label. Do not store below -25°C to avoid freezing.

Sequence specificity

Dpn I recognizes the sequence $\text{G}^{\text{m}}\text{ATC}$ and generates fragments with blunt ends (1). *Dpn* I needs methylation of adenine residues for activity and thus digests only $\text{G}^{\text{m}}\text{ATC}$ sequences containing N^6 -methyladenine (1, 2, 3). Methylation of GATC sequences resulting in N^6 -methyladenine residues is obtained by dam methylase (4). This methylation characteristic distinguishes *Dpn* I from *Mbo* I, which is inhibited by dam methylation, and *Sau* 3A, whose activity is not influenced by dam methylation (5). *Dpn* I is also distinguished from *Mbo* I and *Sau* 3A by the cleavage position (1).

Compatible ends

Dpn I is compatible to any blunt end.

Ischizomers

The enzyme is an isoschizomer to *Bsp* 143 I, *Dpn* II, *Mbo* I, *Nde* II and *Sau* 3A.

Methylation sensitivity

The presence of 5-methylcytosine is only inhibiting (*), when no 6-methyladenine is present.

Storage buffer

10 mM Tris-HCl, 400 mM NaCl, 0.1 mM EDTA, 1 mM Dithiothreitol, 200 $\mu\text{g}/\text{ml}$ Bovine serum albumin, 50% Glycerol (v/v), pH ca. 8.0 (at 4°C).

Suppl Incubation buffer (10x)

330 mM Tris-acetate, 660 mM K-acetate, 100 mM Mg-acetate, 5 mM Dithiothreitol, pH 7.9 (at 37°C), (Δ SuRE/Cut Buffer **A**).

Activity in SuRE/Cut Buffer System

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

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

Incubation temperature

37°C

Unit definition

One unit is the enzyme activity that cleaves 1 μg pBR322 DNA in 1 h at **37°C** in SuRE/Cut buffer **A** in a total volume of 25 μl . Since complete *Dpn* I digestion of pBR322 DNA needs fully methylated GATC sequences < 5% partial bands may be obtained during activity determination.

Typical experiment

Component	Final concentration
DNA	1 μg
10 \times SuRE/Cut Buffer A	2.5 μl
Repurified water	Up to a total volume of 25 μl
Restriction enzyme	1 unit

Incubate at **37°C** for 1 h.

Heat Inactivation

Dpn I can not be 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
116	87	8	0	8	22	27	15

Activity in PCR buffer

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.

Troubleshooting

A critical component is the DNA substrate. Many compounds used in the isolation of DNA e.g. phenol, chloroform, EtOH, SDS, high levels of NaCl, metals (e.g. Hg^{2+} , Mn^{2+}), inhibit or alter recognition specificity of many restriction enzymes. Such compounds should be removed by EtOH precipitation followed by drying, before the DNA is added to the restriction digest reaction. Appropriate mixing of the enzyme is recommended.

☞ Check out the Restrictions Enzymes Frequently Asked Questions at <http://www.roche-applied-science.com/support>.

Quality control

See data label for lot-specific values.

Absence of unspecific endonuclease activities

1 μg pBR322 DNA is incubated for 16 h in 50 μl SuRE/Cut buffer **A** with excess of *Dpn* I. The number of enzyme units which do not change the enzyme-specific pattern is stated under "Endo" printed on the label.

Absence of exonuclease activity

Approx. 5 μg [^3H] labeled calf thymus DNA are incubated with 3 μl *Dpn* I for 4 h at 37°C in a total volume of 100 μl 50 mM Tris-HCl, 10 mM MgCl_2 , 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 under "Exo" as printed on the label).

Ligation and recutting assay

Dpn 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 25°C in 66 mM Tris-HCl, 5 mM MgCl_2 , 5 mM Dithiothreitol, 1 mM ATP, pH 7.5 (at 20°C).

The percentage of ligation and subsequent recutting with *Dpn* I which yields the typical pattern of pBR322 \times *Dpn* I fragments is determined and stated under "Lig" and "Rec" printed on the label.

References

- Lacks, S. & Greenberg, B. (1975) *J. Biol. Chem.* **250**, 4060.
- Geier, G. E. & Modrich, P. (1979) *J. Biol. Chem.* **254**, 1408.
- Lacks, S. & Greenberg, B. (1977) *J. Mol. Biol.* **114**, 153.
- Dreiseikemann, B. et al. (1979) *Biochem. Biophys. Acta* **562**, 418.
- McClelland, M. (1983) *Nucleic Acids Res.* **11**, r169.
- Kessler, C. & Manta, V. (1990) *Gene* **92**, 1-250.
- Hanish, J. and McClelland, M. (1991) "Enzymatic cleavage of a bacterial chromosome at a transposon-inserted rare site" *Nucl. Acids Res.* **19**, 829-832;
- Rebase The Restriction Enzyme Database: <http://rebase.neb.com>
- Benchmark: <http://www.roche-applied-science.com/benchmark>

Ordering Information

Roche Applied Science offers a large selection of reagents and systems for life science research.

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Product	Application	Packsizes	Cat. No.
Restriction Enzymes	DNA restriction digestion	Please refer to website or catalogue	
Rapid DNA Ligation Kit	Ligation of sticky-end or blunt-end DNA fragments in just 5 min at 15–25°C.	Kit (40 DNA ligations)	11 635 379 001
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
Alkaline Phosphatase, shrimp	Dephosphorylation of 5'-phosphate residues from nucleic acids. Heat inactivation: 15 min at 65° C.	1000 U	11 758 250 001
Alkaline Phosphatase (AP), special quality for molecular biology	Dephosphorylation of 5'-phosphate residues from nucleic acids.	1000 U (20 U/μl)	11 097 075 001
Agarose MP	Multipurpose agarose for analytical and preparative electrophoresis of nucleic acids	100 g 500 g	11 388 983 001 11 388 991 001
Agarose LM-MP	Low melting point agarose allows enzymatic manipulations	50 g 100 g	11 441 345 001 11 441 353 001
Agarose Gel DNA Extraction Kit	For the elution of DNA fragments from agarose gels.	1 Kit (max. 100 reactions)	11 696 505 001
High Pure PCR Product Purification Kit	Purification of PCR or enzymatic modification reaction (e.g. restriction digest)	50 purifications 250 purifications	11 732 668 001 11 732 676 001
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) 25 ml (25 vials of 1 ml) 25 ml (1 vial of 25 ml)	03 315 843 001 03 315 932 001 03 315 959 001
BSA, special quality for molecular biology	Maintaining enzyme stability	20 mg (1 ml)	10 711 454 001

Printed Materials

You can view the following manuals on our website:

Laminated Buffer Chart
Lab FAQs "Find a Quick Solution"
Restriction Enzyme FAQs and Ordering Guide
Molecular Weight Markers for Nucleic Acids
Poster "Rec. Sequences of Restriction Enzymes"

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Commonly used bacterial strains

Strain	Genotype
BL21	<i>E. coli</i> B F ⁻ <i>dcm ompT hsdS(r_B⁻ m_B⁻) gal</i> (Studier, F.W. et al (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(lacU169 (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. et al., (1985) <i>Gene</i> 33 , 103.)
JM109	<i>recA1 supE44 endA1 hsdR17 gyrA96 relA1 thi Δ(lac-proAB) F[traD36proAB⁺, lacI^q lacZΔM15]</i> ; (Yanisch-Perron, C. et al., (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⁺, lacI^q lacZΔM15]</i> ; (Yanisch-Perron, C. et al., (1985) <i>Gene</i> 33 , 103.)
K802	<i>supE hsdR gal metB</i> ; (Raleigh, E. et al., (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(hsdRMS) <i>endA1 gyrA96 thi relA1 supE44 F[proAB⁺ lacI^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⁺, lacI^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⁺, lacI^q lacZΔM15 Tn10 (tet^r)</i> ; (Bullock et al., (1987) <i>BioTechniques</i> , 5 , 376.)

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