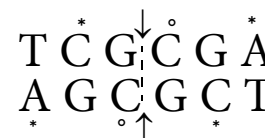


Restriction Endonuclease *Nru* I

From *Nocardia rubra*

Cat. No. 10 776 777 001

1000 units (10 U/μl)



i Version 19
Content version: February 2012

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	<i>Nru</i> I recognizes the sequence TCG/CGA and generates fragments with blunt ends (1).										
Compatible ends	The enzyme generates compatible ends to any blunt end.										
Isoschizomers	<i>Nru</i> I is an isoschizomer to <i>Spo</i> I.										
Methylation sensitivity	<i>Nru</i> I is inhibited by overlapping dam-methylation as indicated (*). The presence of 5-methylcytosine is as indicated; inhibiting (*) or not inhibiting (°).										
Storage buffer	20 mM Tris-HCl, 100 mM NaCl, 0.1 mM EDTA, 10 mM 2-Mercaptoethanol, 0.05% Polydocanol, 50% Glycerol (v/v), pH approx. 8.0 (at 4°C).										
Suppl. Incubation buffer (10×)	100 mM Tris-HCl, 1 M NaCl, 50 mM MgCl ₂ , 10 mM 2-Mercaptoethanol, pH 8.0 (at 37°C), (= SuRE/Cut Buffer B)										
Activity in SuRE/Cut Buffer System	Bold face printed buffer indicates the recommended buffer for optimal activity:										
	<table border="1"> <thead> <tr> <th>A</th> <th>B</th> <th>L</th> <th>M</th> <th>H</th> </tr> </thead> <tbody> <tr> <td>10-25%</td> <td>100%</td> <td>0-10%</td> <td>10-25%</td> <td>75-100%</td> </tr> </tbody> </table>	A	B	L	M	H	10-25%	100%	0-10%	10-25%	75-100%
A	B	L	M	H							
10-25%	100%	0-10%	10-25%	75-100%							
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 B . 1 μg pBR322 DNA is digested completely by approx. 2 units of <i>Nru</i> I on account of the larger number of cleavage sites per μg of pBR322 DNA.										
Typical experiment	<table border="1"> <thead> <tr> <th>Component</th> <th>Final concentration</th> </tr> </thead> <tbody> <tr> <td>DNA</td> <td>1 μg</td> </tr> <tr> <td>10 × SuRE/Cut Buffer B</td> <td>2.5 μl</td> </tr> <tr> <td>Repurified water</td> <td>Up to a total volume of 25 μl</td> </tr> <tr> <td>Restriction enzyme</td> <td>1 unit</td> </tr> </tbody> </table> <p>Incubate at 37°C for 1 h.</p>	Component	Final concentration	DNA	1 μg	10 × SuRE/Cut Buffer B	2.5 μl	Repurified water	Up to a total volume of 25 μl	Restriction enzyme	1 unit
Component	Final concentration										
DNA	1 μg										
10 × SuRE/Cut Buffer B	2.5 μl										
Repurified water	Up to a total volume of 25 μl										
Restriction enzyme	1 unit										
Heat inactivation	<i>Nru</i> I can be heat-inactivated by heating to 65°C for 15 min.										

Number of cleavage sites on different DNAs (2):

λ	Ad2	SV40	Φ X174	M13mp7	pBR322	pBR328	pUC18
5	5	0	2	0	1	2	0

PFGE tested

Nru I has been tested in Pulsed-Field Gel Electrophoresis (test system bacterial chromosomes). For cleavage of genomic DNA (*E. coli* C600) embedded in agarose for PFGE analysis 10 units of enzyme/μg DNA and 4 h incubation time are recommended.

Activity in PCR buffer

Relative activity in PCR mix (Taq DNA Polymerase buffer) is 75%. 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.

Ligation and recutting assay

Nru 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) resulting in >90% recovery of 1 μg pBR322 DNA fragments. Subsequent re-cutting with *Nru* I yields > 80% of the typical pattern of pBR322 DNA × *Nru* I fragments

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.

Quality control

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

Absence of unspecific endonuclease activities

1 μg λDNA is incubated for 16 h in 25 μl SuRE/Cut buffer B with excess of *Nru* 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 *Nru* 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. Under these conditions, no release of radioactivity is detectable, as stated in the certificate of analysis.

References

- 1 Comb, D. G. & Schildkraut, I., unpublished results.
- 2 Kessler, C. & Manta, V. (1990) *Gene* **92**, 1-248.
- 3 Rebase The Restriction Enzyme Database: <http://rebase.neb.com>
- 4 Benchmate: <http://www.roche-applied-science.com/benchmark>

Ordering Information

Roche Applied Science offers a large selection of reagents and systems for life science research. For a complete overview of related products and manuals, please visit and bookmark our home page, www.roche-applied-science.com, and our Special Interest Sites, including "Mapping & Cloning": <http://www.restriction-enzymes.com>.

The convenient RE Finder Program located on our Bench Mate website, <http://www.roche-applied-science.com/benchmate> helps you identify the enzymes that will cut your DNA sequence, and displays the names and recognition sequences of enzymes and isoschizomers as well as links to detailed information (e.g. instructions for use) of the selected restriction enzyme.

Product	Application	Packsize	Cat. No.
Restriction Enzymes	DNA restriction digestion	Please refer to website or catalogue	
Rapid DNA Ligation Kit	Ligation of sticky- or blunt-ended DNA fragments in just 5 min at +15 to +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
rAPid Phosphatase	Dephosphorylation of 5'-phosphate residues from nucleic acids	1000 U 5000 U	04 898 133 001 04 898 141 001
rAPid Dephos and Ligation Kit	Dephosphorylation of nucleic acids.	40 reactions 160 reactions	04 898 117 001 04 898 125 001
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 LE	Separation of nucleic acids in the range 0.2 - 1.5 kbp	100 g 500 g	11 685 660 001 11 685 678 001
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:

Lab FAQs "Find a Quick Solution"
Restriction Enzyme Ordering Guide
Molecular Weight Markers for Nucleic Acids

Changes to previous version

Update of quality control.

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Regulatory Disclaimer

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

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. <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(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. <i>et al.</i> , (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. <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⁺, lacI^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⁺, Δ(hsdRMS) endA1 gyrA96 thi relA1 supE44 F[proAB⁺ lacI^q lacZΔM15 Tn10 (tet^r)</i> ; (Greener, A. (1990) <i>Strategies</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 <i>et al.</i> , (1987) <i>BioTechniques</i> , 5 , 376.)

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Roche Diagnostics GmbH
Roche Applied Science
68298 Mannheim
Germany