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

Restriction Endonuclease Nhe I

From *Neisseria mucosa*

Cat. No. 10 885 843 001 200 units (10 U/µl)
Cat. No. 10 885 851 001 1,000 units (10 U/µl)
Cat. No. 10 885 860 001 1,500 units , high concentration (40 U/µl)

Version 20
Content version: November 2012
Store at −15 to −25°C

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

Sequence specificity *Nhe* I recognizes the sequence G/CTAGC and generates fragments with 5´-cohesive termini (1, 2).

Compatible ends *Nhe* I generates compatible ends to *Avr* II, *Spe* I, and *Xba* I.

Enzyme with compatible ends	Recognition sequence	New sequence if *Nhe* I is ligated to enzyme with compatible ends		Enzyme that can cut this new sequence
		Nhe I - Enzyme	Enzyme – *Nhe* I	
***Avr* II**	C/CTAGG	G<u>C/TAG</u>G	C<u>C/TAG</u>C	*Bfa* I, *Mae* I
***Nhe* I**	**G/CTAGC**	**G/CTAGC**	**G/CTAGC**	***Nhe* I** + Isoschizomers
***Spe* I**	A/CTAGT	G<u>C/TAG</u>T	A<u>C/TAG</u>C	*Bfa* I, *Mae* I
***Xba* I**	T/CTAGA	G<u>C/TAG</u>A	T<u>C/TAG</u>C	*Bfa* I, *Mae* I

Isoschizomers The enzyme is not known to have isoschizomers.

Methylation sensitivity *Nhe* I is inhibited by the presence of 5´-methylcytosine in the sequence G/CTAG*C.

Storage buffer 20 mM Tris-HCl, 100 mM NaCl, 0.1 mM EDTA, 10 mM 2-Mercaptoethanol, 0.2% Polydocanol, 0.01% Gelatine, 50% Glycerol (v/v), pH approx. 7.8 (at 4°C).

Suppl. Incubation buffer (10x) 100 mM Tris-HCl, 500 mM NaCl, 100 mM $MgCl_2$, 10 mM Dithioerythritol, pH 7.5 (at 37°C), (≙ SuRE/Cut Buffer **M**).

Activity in SuRE/Cut Buffer System Bold face printed buffer indicates the recommended buffer for optimal activity:

A	B	L	**M**	H
100%	25-50%	100%	**100%**	10-25%

Incubation temperature **37°C**

Unit definition One unit is the enzyme activity that completely cleaves 1 µg λ x *Eco* RI DNA in 1 h **at 37°C** in a total volume of 25 µl SuRE/Cut buffer **M**. 1 µg pBR322 DNA is digested completely by approx. 8 units of *Nhe* I because of the larger number of cleavage sites per µg of pBR322 DNA as compared to λDNA.

Typical experiment

Component	**Final concentration**
DNA	1 µg
10× SuRE/Cut Buffer **M**	2.5 µl
Sterile double-dist. water	Up to a total volume of 25 µl
Restriction enzyme	1 unit

Incubate at **37°C** for 1 h.

Heat Inactivation *Nhe* 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
1	4	0	0	0	1	1	0

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.

Ligation and recutting assay λ x *Eco* RI/*Nhe* I fragments obtained by complete digestion of 1 µg λ × *Eco* RI/*Nhe* I DNA are ligated with 1 U T4 DNA Ligase in a volume of 10 µl by incubation for 16 h at 4°C in 66 mM Tris-HCl, 5 mM $MgCl_2$, 5 mM dithiothreitol, 1 mM ATP, pH 7.5 (at 20°C) resulting in >90 % recovery of 1 µg λDNA × *Eco* RI/*Nhe* I fragments.
Subsequent re-cutting with *Eco* RI/*Nhe* I yields > 80% of the typical pattern of λDNA × *Eco* RI/*Nhe* I fragments.

Troubleshooting A critical component is the DNA substrate.
Many compounds used in the isolation of DNA such as phenol, chloroform , EtOH, SDS, high levels of NaCl, metals (*e.g.*, Hg^{2+}, Mn^{2+}), inhibit or alter recognition specifity of many restriction enzymes.
ISuch 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.

Quality control

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

Absence of unspecific endonuclease activities 1 µg λ × *Eco* RI DNA is incubated for 16 h in 50 µl SuRE/Cut buffer M with excess of *Nhe* 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 [^{3}H] labeled calf thymus DNA are incubated with 3 µl *Nhe* 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. Under these conditions, no release of radioactivity is detectable, as stated in the certificate of analysis.

References

1 Comb, D. G. & Schildkraut, I., unpublished observations
2 Kessler, C. & Manta, V. (1990) *Gene* **92,** 1–250.
3 Rebase The Restriction Enzyme Database: http://rebase.neb.com
4 Benchmate: http://www.roche-applied-science.com/benchmate

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 homepage, 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	Pack Size	Cat. No.
Restriction Enzymes	DNA restriction digestion.	Please refer to website or catalog	
T4 DNA Ligase	Ligation of sticky- and blunt-ended DNA fragments.	100 U 500 U (1 U/µl) 500 U (5 U/µl)	10 481 220 001 10 716 359 001 10 799 009 001
Rapid DNA Dephos & Ligation Kit	Upgrade from the Rapid DNA Ligation Kit for fast and efficient DNA dephosphorylation and ligation of sticky- or blunt-ended DNA fragments.	40 reactions 160 reactions	04 898 117 001 04 898 125 001
rAPid Alkaline Phosphatase	Dephosphorylation of 5´-phosphate residues from nucleic acids.	1,000 U 5,000 U	04 898 133 001 04 898 141 001
Rapid DNA Ligation Kit	Ligation of nucleic acids.	Kit (40 DNA ligations)	11 635 379 001
Alkaline Phosphatase (AP), special quality for molecular biology	Dephosphorylation of 5´-phosphate residues from nucleic acids.	1,000 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 Star activity information removed.

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Commonly used bacterial strains

Strain	Genotype
BL21	*E. coli B F⁻ dcm ompT hsdS(r_B- m_B-) gal* (Studier, F.W. *et al* (1986) *J. Mol. Biol.*, **189,** 113.)
C600[e]	*supE44 hsdR2 thi-1 thr-1 leuB6 lacY1 tonA21*; (Hanahan, D. (1983) *J. Mol. Biol.* **166**, 557.)
DH5α	*supE44* Δ(*lac*U169 (ϕ80d*lacZ*ΔM15) *hsd*R17 *rec*A1 *end*A1 *gyr*A96 *thi*-1 *rel*A1; (Hanahan, D. (1983) *J. Mol. Biol.* **166**, 557.)
HB101	*supE44 hsdS20 recA13 ara-14 proA2 lacY1 galK2 rpsL20 xyl-5 mtl-1*; (Hanahan, D., (1983) *J. Mol. Biol.* **166**, 557.)
JM108	*recA1 supE44 endA1 hsdR17 gyrA96 relA1 thi* Δ(*lac-pro*AB); (Yanisch- Perron, C. *et al.,* (1985) *Gene* **33***,* 103.)
JM109	*recA1 supE44 endA1 hsdR17 gyrA96 relA1 thi* Δ(*lac-pro*AB) F'[*traD36proAB*$^+$, *lac*I^q *lacZ*ΔM15]; (Yanisch- Perron, C. *et al.,* (1985) *Gene* **33***,* 103.)
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) *Gene* **33**, 103.)
K802	*supE hsdR gal metB;* (Raleigh, E. et al., (1986) Proc.Natl. Acad.Sci USA, 83, 9070.; Wood, W.B. (1966) *J. Mol. Biol.,* **16**, 118.)
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) *Stratagies,* **3**, 5.)
TG1	*supE hsd* Δ*5 thi* Δ(*lac-pro*AB) F'[*traD36proAB*$^+$, *lacI^q lacZ*ΔM15]; (Gibson, T.J. (1984) PhD Theses. Cambridge University, U.K.)
XL1-Blue[r]	*supE44 hsdR17 recA1 endA1 gyrA46 thi relA1 lac* F'[*proAB*$^+$, *lacI^q lacZ*ΔM15 Tn10 *(tet^r)*]; (Bullock *et al.,* (1987) *BioTechniques*, 5, 376.)

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