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

Restriction Endonuclease *Hind* III

From *Haemophilus influenza* Rd com-10

Cat. No. 10 656 313 001 5000 U (10 U/µl)
Cat. No. 10 656 321 001 10 000 U (10 U/µl)
Cat. No. 10 798 983 001 10 000 U, high concentration (40 U/µl)
Cat. No. 11 274 040 001 50 000 U, high concentration (40 U/µl)

Version 21
Content version: June 2017
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.
Note: Product is shipped on dry ice.

Sequence specificity
Hind III recognizes the sequence A/AGCTT and generates fragments with 5´-cohesive termini (1).

Compatible ends
The enzyme is not known to have compatible ends.

Isoschizomers
The enzyme is not known to have isoschizomers.

Methylation sensitivity
Hind III is inhibited by 6-methyladenine and 5-methylcytosine as indicated (*). Hydroxymethylcytosine is also inhibiting. The presence of 6-methyladenine at the most central A-residue is not inhibiting (°).

Storage buffer
10 mM Tris-HCl, 250 mM NaCl, 0.1 mM EDTA, 1 mM dithioerythritol, 0.01% polydocanol, 60% glycerol (v/v), pH approx. 7.5 (at 4°C).

Incubation buffer (10×, included)
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:

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

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 SuRE/Cut buffer in a total volume of 25 µl.

Typical experiment

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 U

Incubate at **37°C** for 1 h.

Heat inactivation
Hind III is inactivated by 15 min incubation at 65°C (tested up to 100 U/µg DNA).

Number of cleavage sites on different DNAs (2):

λ	Ad2	SV40	Φ X174	M13mp7	pBR322	pBR328	pUC18
6	12	6	0	0	1	1	1

Activity in PCR buffer
Relative activity in PCR mix (Taq DNA Polymerase buffer) is **10%**. 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
Hind III fragments obtained by complete digestion of 1 µg λDNA 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 >95 % recovery of 1 µg λDNA × *Hind* III fragments.
Subsequent re-cutting with *Hind* III yields > 95% of the typical pattern of λDNA × *Hind* III fragments.

Troubleshooting
A critical component is the DNA substrate. Many compound used in the isolation of DNA, *e.g.* phenol, chloroform, ethanol, SDS, high concentrations of NaCl, metals (*e.g.* Hg^{2+}, Mn^{2+}) 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. Mix the enzyme before use.

Star activity
Hind III exhibits star activity under non-optimal conditions. The recognition specificity of *Hind* III is altered by addition of increasing amounts of hydrophobic reagents and glycerol to the incubation mixture. This activity is the so-called star activity.

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

Absence of unspecific endonuclease activities
1 µg λDNA or T7 DNA is incubated for 16 h in 50 µl SuRE/Cut buffer B with excess of *Hind* III. The number of enzyme units which do not change the enzyme-specific pattern is in the certificate of analysis.

Absence of exonuclease activity
Approx. 5 µg [^{3}H] labeled calf thymus DNA are incubated with 3 µl *Hind* III for 4 h at 37°C in a total volume of 100 µl 50 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM dithiolerythritol, pH approx. 7.5. Under these conditions, no release of radioactivity is detectable, as stated in the certificate of analysis.

References

1 Old, R. *et al.* (1975) *J. Mol. Biol.* **92**, 331..
2 Kessler, C. & Manta, V. (1990) *Gene* **92,** 1–248.
3 Rebase The Restriction Enzyme Database: http://rebase.neb.com

Ordering Information

Product	Application	Packsize	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) 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

Changes to previous version Editorial changes

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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*lac*ZΔM15) *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} *lac*ZΔM15]; (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 Δ5 thi* Δ(*lac-pro*AB) F'[*traD36proAB*$^{+}$, *lac*I^{q} *lac*ZΔM15]; (Gibson, T.J. (1984) PhD Theses. Cambridge University, U.K.)
XL1-Blue^{r}	*supE44 hsdR17 recA1 endA1 gyrA46 thi relA1 lac* F'[*proAB*$^{+}$, *lac*I^{q} *lac*ZΔM15 Tn10 *(tet^{r})*]; (Bullock *et al.,* (1987) BioTechniques, 5, 376.)

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