

# Restriction Endonuclease *Hinf* I

From *Haemophilus influenzae* R<sub>f</sub>

**Cat. No. 10 779 652 001** 1000 units (10 U/μl)  
**Cat. No. 10 779 679 001** 5000 units (10 U/μl)  
**Cat. No. 11 274 082 001** 20 000 units, high concentration (40 U/μl)



**Version 18**  
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 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:** The product is shipped on dry ice.

**Sequence specificity** *Hinf* I recognizes the sequence G/A/N/T/C and generates fragments with 5'-cohesive termini (1).

**Compatible ends** The enzyme is not known to generate compatible ends.

**Isoschizomers** The enzyme is not known to have isoschizomers.

**Methylation sensitivity** *Hinf* I is sensitive to the presence of 6-methyladenine as indicated (\*). 5-methylcytosine in the 3'- position does not prevent cleavage, but the presence of 5-hydroxymethylcytosine does (°)

**Storage buffer** 20 mM Tris-HCl, 100 mM NaCl, 50 mM KCl, 0.1 mM EDTA, 10 mM 2-Mercaptoethanol, 0.01% Gelatine, 0.05% Polydocanol (v/v), 50% Glycerol (v/v), pH approx. 7.5 (at 4°C).

**Incubation buffer, (10x, included)** 500 mM Tris-HCl, 1 M NaCl, 100 mM MgCl<sub>2</sub>, 10 mM Dithioerythritol, pH 7.5 (at 37°C), (= 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
100%	100%	50-75%	75-100%	<b>100%</b>

**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 in SuRE/Cut buffer **H**. 1 μg pBR322 DNA is digested completely by ca. 2 units of *Hinf* I on account of the larger number of cleavage sites per μg pBR322 DNA as compared to λDNA.

**Typical experiment**

Component	Final concentration
DNA	1 μg
10 × SuRE/Cut Buffer <b>H</b>	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** The enzyme cannot 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
148	72	10	21	26	10	10	6

**Activity in PCR buffer** Relative activity in PCR mix (Taq DNA Polymerase buffer) is **50%**. The PCR mix contained λ target DNA, primers, 10 mM Tris-HCl (pH 8.3, 20°C), 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 200 μM dNTPs, 2.5 U Taq DNA polymerase. The mix was subjected to 25 amplification cycles.

**Ligation and recutting assay** *Hinf* I fragments obtained by complete digestion of 1 μg λDNA 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 MgCl<sub>2</sub>, 5 mM dithioerythritol, 1 mM ATP, pH 7.5 (at 20°C) resulting in >95 % recovery of 1 μg λDNA × *Hinf* I fragments. Subsequent re-cutting with *Hinf* I yields > 95% of the typical pattern of λDNA × *Hinf* I fragments

**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<sup>2+</sup>, Mn<sup>2+</sup>) 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.

**Quality control** Lot-specific certificates of analysis are available at [www.lifescience.roche.com/certificates](http://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 *Hinf* 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 [<sup>3</sup>H] labeled calf thymus DNA are incubated with 3 μl *Hinf* I for 4 h at 37°C in a total volume of 100 μl 50 mM Tris-HCl, 10 mM MgCl<sub>2</sub>, 1 mM dithioerythritol, pH approx. 7.5. Under these conditions, no release of radioactivity is detectable, as stated in the certificate of analysis.

**References**

- Gingeras, T. R. *et al.* (1981) *Nucleic Acids Res.* **9**, 4525.
- Kessler, C. & Manta, V. (1990) *Gene* **92**, 1-250.
- Bassing, C.H. *et al.* (1992) *Gene* **113**, 83-88;
- Chandrasegaran, S. *et al.* (1988) *Gene* **70**, 387-392.
- Petronzio, T. and Schildkraut, I. (1990) *Nucl. Acids Res.* **18**, 3666.
- Shimizu, Y. *et al.* (1983) *Nature* **302**, 587-590.
- Skoglund, C.M. *et al.* (1990) *Gene* **88**, 1-5.
- Rebase The Restriction Enzyme Database: <http://rebase.neb.com>

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

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