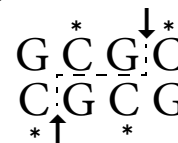


Restriction Endonuclease Cfo I (Hha I)

From *Clostridium formicoaceticum*

Cat. No. 10 688 541 001

1000 units (10 U/μl)



Version 17

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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.

Sequence specificity Cfo I recognizes the sequence GCG/C and generates fragments with 3'-cohesive termini (1,2).

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

Isoschizomers Cfo I is an isoschizomer of Hha I, Hin 6 I and Hin P1 I.

Methylation sensitivity Cfo I is inhibited by the presence of 5-methylcytosine, as indicated (*). It is also inhibited by the simultaneous presence of 5-hydroxymethylcytosine at both C-residues.

Storage buffer 20 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, 10 mM 2-Mercaptoethanol, 0.02% Polydocanol, 0.1 mM PMSF, 50% Glycerol (v/v), pH approx. 8.0 (at 4°C).

Suppl. Incubation buffer (10x) 100 mM Tris-HCl, 100 mM MgCl₂, 10 mM Dithiothreitol, pH 7.5 (at 37°C), (Δ SuRE/Cut Buffer L).

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

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

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 L in a total volume of 25 μl. 1 μg pBR322 DNA is digested completely by ca. 1.75 units of Cfo I on account of the larger number of cleavage sites per μg of pBR322 DNA.

Typical experiment

Component	Final concentration
DNA	1 μg / 25 μl
10x SuRE/Cut Buffer L	2.5 μl
Sterile redist. water	Up to a total volume of 25 μl
Restriction enzyme	1 unit

Incubate at 37°C for 1 h.

Heat Inactivation It was not determined if this enzyme can be 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
215	375	2	18	25	31	25	17

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 MgCl₂, 200 μM dNTPs, 2.5 U Taq DNA polymerase. The mix was subjected to 25 amplification cycles.

Ligation and recutting assay Cfo I fragments obtained by complete digestion of 1 μg λDNA are ligated with 1.0 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 MgCl₂, 1 mM dithioerythritol, 1 mM ATP, pH 7.5 (at 20°C) resulting in >95% recovery of 1 μg λDNA fragments. Subsequent re-cutting with Cfo I yields > 95% of the typical pattern of λDNA × Cfo 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.lifescience.roche.com/certificates.

Absence of unspecific endonuclease activities 1 μg λDNA is incubated for 16 h in 50 μl SuRE/Cut buffer L with excess of Cfo 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 g [³H] labeled calf thymus DNA are incubated with 3 μl Cfo 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 Makula, R. A. & Meagher, R. B. (1980) *Nucleic Acids Res.* **8**, 3125
- 2 Kessler, C. & Manta, V. (1990) *Gene* **92**, 1-250.
- 3 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 ⁻ <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(lacU)169 (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⁺, lac^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⁺, lac^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</i> , Δ(<i>hsdRMS</i>) <i>endA1 gyrA96 thi relA1 supE44 F[proAB⁺ lac^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⁺, lac^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⁺, lac^q lacZΔM15 Tn10 (tet^r)</i> ; (Bullock <i>et al.</i> , (1987) <i>BioTechniques</i> , 5 , 376.)

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