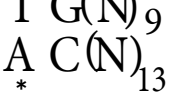
For life science research only.
Not for use in diagnostic procedures.

Restriction Endonuclease Fok I

From *Floavobacterium okeanokoites*

Cat. No. 11 004 816 001 100 units (1- 5 U/µl)



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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
Fok I recognizes the sequence $GGATG(N)_{9/13}$ and generates fragments with 5'-cohesive termini (1). *Fok* I belongs to the class II S restriction endonucleases which cleave double-stranded DNA at precise distances from their recognition sequences. *Fok* I can be used as a universal restriction enzyme to cleave practically any sequence by combination with a DNA adaptor oligonucleotide to mediate novel sequence specificities.

Compatible ends
Fok I has no compatible ends to other known restriction enzymes.

Isoschizomers
The enzyme is not known to have isoschizomers.

Methylation sensitivity
Fok I is inhibited by the presence of 6-methyladenine, as indicated (*).

Storage buffer
10 mM Tris-HCl, 100 mM KCl, 0.1 mM EDTA, 1 mM Dithiothreitol, 0.15% Triton X-100, 50% Glycerol, (v/v), 100 µg/ml bovine serum albumin; pH approx. 7.5 (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	50-75%	75-100%	**100%**	25-50%

Incubation temperature
37°C

Unit definition
One unit is the enzyme activity that completely cleaves 1 µg pBR322 DNA in 1 h **at 37° C** in a total volume of 25 µl incubation buffer M.

Typical experiment

Component	Final concentration
DNA	1 µg
10 × SuRE/Cut Buffer **M**	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 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
150	78	11	8	4	12	11	5

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^{2+}, Mn^{2+}), inhibit or alter recognition specifity 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.

Absence of unspecific endonuclease activities
1 µg pBR322, DNA is incubated for 16 h in 50 µl incubation buffer with excess of *Fok* 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 *Fok* 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. The release of radioactivity is calculated as a percentage value of liberated to input radioactivity per unit of enzyme (stated in the certificate of analysis).

Ligation and recutting assay
Fok 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 $MgCl_2$, 5 mM Dithiothreitol, 1 mM ATP, pH 7.5 (at 20° C).
The percentage of ligation and subsequent recutting with Fok I yielding the typical pattern of pBR322-*Fok* I fragments are determined and stated in the certificate of analysis.

References

1 Sugisaki, H. & Kanazawa, S. (1981) *Gene* **16,** 73-78.
2 Szybalski, W. (1985) *Gene* **40,** 169-173.
3 Podhajska, A. J. & Szybalski, W. (1985) *Gene* **40,** 175-181.
4 Kessler & Höltke (1986) *Gene* **47**, 1–53
5 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*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*$^+$, *lacI*^{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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Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany