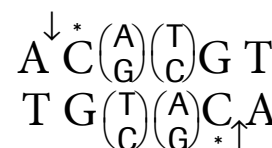


# Restriction Endonuclease *Afl* III

From *Anabaena flos-aquae*

Cat. No. 11 209 183 001 100 U (5 U/μl)



Version 09

Content version: February 2016

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** *Afl* III recognizes the sequence A/C  $\begin{pmatrix} A \\ G \end{pmatrix} \begin{pmatrix} T \\ C \end{pmatrix}$  GT and generates fragments with 5'-cohesive ends (1).

**Compatible ends** The enzyme generates compatible ends to *Dsa* I, *Nco* I and *Rca* I.

Enzyme with compatible ends	Recognition sequence	New sequence if <i>Afl</i> III is ligated to enzyme with compatible ends		Enzyme that can cut this new sequence
		<i>Afl</i> III - Enzyme	Enzyme - <i>Afl</i> III	
<i>Afl</i> III	A/C(A,G)(T,C)GT	A/C(A,G)(T,C)GT	A/C(A,G)(T,C)GT	<i>Bsp</i> LU11 I
<i>Dsa</i> I	C/C(A,G)(C,T)GG	A/C(A,G)(C,T)GG	C/C(AG)(T,C)GT	<i>Mae</i> II, <i>Mvn</i> I,
<i>Nco</i> I	C/CATGG	A/CATGG	C/C(AG)(T,C)GT	<i>Hpa</i> II, <i>Msp</i> I
<i>Rca</i> I	T/CATGA	A/CATGA	T/C(AG)(T,C)GT	<i>Mae</i> II, <i>Mvn</i> I,

**Isoschizomers** *Afl* III is not known to have isoschizomers.

**Methylation sensitivity** *Afl* III is inhibited by the presence of 5-methylcytosine as indicated (\*).

**Storage buffer** 10 mM Tris-HCl, 500 mM NaCl, 0.1 mM EDTA, 1 mM Dithiothreitol, 200 μg/ml BSA, 50% Glycerol, pH 7.4 (at 25°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
50-75%	75-100%	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 hr at **37°C** in total volume of 25 μl incubation buffer **H**.

**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 U

Incubate at **37°C** for 1 h.

**Heat inactivation** *Afl* III is not heat-inactivated by incubation at 65°C. 50% activity remain after 20 min incubation at 65°C.

**Number of cleavage sites on different DNAs (2):**

λ	Ad2	SV40	Φ X174	M13mp7	pBR322	pBR328	pUC18
20	25	0	2	3	1	14	1

**Activity in PCR buffer** Relative activity in PCR mix (Taq DNA Polymerase buffer) is **20%**. 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.

**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., Hg<sup>2+</sup>, Mn<sup>2+</sup>) 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.roche-applied-science.com/certificates](http://www.roche-applied-science.com/certificates).

**Absence of unspecific endonuclease activities** 1 μg λDNA is incubated for 16 h in 50 μl incubation buffer with excess of *Afl* III. 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 *Afl* III 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. 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** *Afl* III 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 Dithiothreitol, 1 mM ATP, pH 7.5 (at 20°C). The percentage of ligation and subsequent recutting with *Afl* III yielding the typical pattern of λ × *Afl* III fragments are determined and stated in the certificate of analysis.

**References**

- Whitehead, P. R. & Brown, N. L. (1985) *J. Gen. Microbiol.* **131**, 951-958.
- Kessler, C. & Höltke, H.-J. (1986) *Gene* **47**, 1-153.
- van Heuverswyn, H. & Fiers, W. (1980) *Gene* **9**, 195-203.
- Rebase The Restriction Enzyme Database: <http://rebase.neb.com>
- Benchmark: <http://biochem.roche.com/benchmark>

## Ordering Information

Product	Application	Packsizes	Cat. No.
Restriction Enzymes	DNA restriction digestion	Please refer to website or catalogue	
Rapid DNA Ligation Kit	Ligation of sticky- or blunt-ended DNA fragments in just 5 min at 15 - 25 °C.	Kit (40 DNA ligations)	11 635 379 001
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
rAPid Phosphatase	Dephosphorylation of 5'-phosphate residues from nucleic acids	1000 U 5000 U	04 898 133 001 04 898 141 001
rAPid Dephos and Ligation Kit	Dephosphorylation of nucleic acids.	40 reactions 160 reactions	04 898 117 001 04 898 125 001
Alkaline Phosphatase (AP), special quality for molecular biology	Dephosphorylation of 5'-phosphate residues from nucleic acids.	1000 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	Specialty 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

### Changes to previous version

New storage buffer

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### Regulatory Disclaimer

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

## 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(lacU169 (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, Δ(hsdRMS) 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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