Thermo SCIENTIFIC

PRODUCT INFORMATION

AarI

#ER1581 25 U

Lot: ___ **Expiry Date: _**

5'...**C A C C T G C(N)$_4$**↓...3'

3'...**G T G G A C G(N)$_8$**↑...5'

Concentration: 2 U/µL

Supplied with: 1 mL of 10X Buffer AarI
1 mL of 10X Buffer Tango
25 µL of 50X oligonucleotide (0.025 mM)

Store at -20°C

In total 4 vials. BSA included

www.thermoscientific.com/onebio

RECOMMENDATIONS

[1X Buffer AarI] + oligonucleotide* (for 100% AarI digestion)
[10 mM Bis-Tris Propane-HCl (pH 6.5 at 37°C), 10 mM $MgCl_2$,100 mM KCl, 0.1 mg/mL BSA] + 0.5 µM of oligonucleotide.

Incubation temperature
37°C.

Unit Definition
One unit is defined as the amount of AarI at which no change in the fragmentation pattern is observed with further increase of enzyme. 1 µg of lambda DNA is incubated with the enzyme for 1 hour at 37°C in 50 µL of recommended reaction buffer. The cleavage of DNA by AarI is never complete.

Dilution
Dilute with Dilution Buffer (#B19): 10 mM Tris-HCl (pH 7.4 at 25°C), 100 mM KCl, 1 mM EDTA, 1 mM DTT, 0.2 mg/mL BSA and 50% glycerol.

Double Digests
Thermo Scientific Tango Buffer is provided to simplify buffer selection for double digests. 98% of Thermo Scientific restriction enzymes are active in a 1X or 2X concentration of Tango™ Buffer. Please refer to www.thermoscientific.com/doubledigest to choose the best buffer for your experiments.
1X Tango Buffer: 33 mM Tris-acetate (pH 7.9 at 37°C), 10 mM magnesium acetate, 66 mM potassium acetate, 0.1 mg/mL BSA.

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* AarI exhibits DNA site preferences. Plasmid DNAs containing single AarI sites are resistant to cleavage. Digestion of slowly cleaved and resistant sites can be significantly enhanced by the addition of a 0.5 µM oligonucleotide containing AarI recognition sequence.

Storage Buffer

AarI is supplied in: 10 mM potassium phosphate (pH 7.4 at 25°C), 100 mM KCl, 1 mM EDTA, 1 mM DTT, 0.2 mg/mL BSA and 50% glycerol.

Recommended Protocol for Digestion

- Add:

nuclease-free water	16 µL
10X Buffer AarI	2 µL
DNA (0.5-1 µg/µL)	1 µL
50X oligonucleotide (0.025 mM)	0.4 µL
AarI	0.5-2 µL**

- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours**.

The digestion reaction may be scaled either up or down.

Recommended Protocol for Digestion of PCR Products

- Add:

PCR reaction mixture	10 µL (~0.1-0.5 µg of DNA)
nuclease-free water	18 µL
10X Buffer AarI	2 µL
50X oligonucleotide (0.025 mM)	0.6 µL
AarI	1-2 µL**

- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours**.

** *See* Star Activity.

Thermal Inactivation

AarI is inactivated by incubation at 65°C for 20 min.

ENZYME PROPERTIES

Enzyme Activity in Thermo Scientific REase Buffers, %

AarI$_{+oligo}$	B$_{+oligo}$	G$_{+oligo}$	O$_{+oligo}$	R$_{+oligo}$	Tango$_{+oligo}$	2X Tango$_{+oligo}$
100	NR	NR	0-20	0-20	NR	50-100

NR – buffer is not recommended, because of high star activity.

Star Activity

An excess of AarI (1 U/µg DNA x 16 hours) may result in star activity.

Methylation Effects on Digestion

Dam: never overlaps – no effect.
Dcm: never overlaps – no effect.
CpG: may overlap – cleavage impaired.
EcoKI: never overlaps – no effect.
EcoBI: may overlap – effect not determined.

Stability during Prolonged Incubation

A minimum of 1 unit of the enzyme is required for digestion of 1 µg of lambda DNA in 16 hours at 37°C.

Digestion of Agarose-embedded DNA

A minimum of 5 units of the enzyme is required for digestion of 1 µg of agarose-embedded lambda DNA in 16 hours.

Number of Recognition Sites in DNA

λ	ΦX174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
12	0	0	0	0	0	0

For **CERTIFICATE OF ANALYSIS** *see* back page

Note

- For cleavage with AarI at least two copies of its recognition sequence are required.
- AarI may remain associated with the cleaved DNA. This may cause DNA band shifting during electrophoresis. To avoid atypical DNA band patterns, use the 6X DNA Loading Dye&SDS Solution (#R1151) for sample preparation or heat the digested DNA in the presence of SDS prior to electrophoresis.

CERTIFICATE OF ANALYSIS

Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after a 10-fold overdigestion with AarI (10 U/µg lambda DNA x 1 hour) (*see* Star Activity).

Ligation and Recleavage (L/R) Assay

The ligation and recleavage assay was replaced with LO test after validating experiments showed LO test ability to trace nuclease and phosphatase activities with sensitivity that is higher than L/R by a factor of 100.

Labeled Oligonucleotide (LO) Assay

No detectable degradation of single-stranded or double-stranded labeled oligonucleotides occurred during incubation with 5 units of AarI for 4 hours.

Blue/White (B/W) Cloning Assay

The B/W assay was replaced with LO test after validating experiments showed LO test ability to detect nuclease and phosphatase activities with sensitivity that equals to that of B/W test.

Quality authorized by:  Jurgita Zilinskiene