

## PRODUCT INFORMATION

# AarI

**#ER1582** 125 U

**Lot:** \_\_\_\_ **Expiry Date:** \_\_

5'...C A C C T G C(N)<sub>4</sub> ↓...3'

3'...G T G G A C G(N)<sub>8</sub> ↑...5'

Concentration: 2 U/μL

Supplied with: 1 mL of 10X Buffer AarI

1 mL of 10X Buffer Tango

2 x 25 μL of 50X oligonucleotide (0.025 mM)

**Store at -20°C**



In total 5 vials.

BSA included

[www.thermoscientific.com/onebio](http://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<sub>2</sub>, 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](http://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.

\* 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

Aarl 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 Aarl	2 µL
DNA (0.5-1 µg/µL)	1 µL
50X oligonucleotide (0.025 mM)	0.4 µL
Aarl	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 (DNA)	10 µL (~0.1-0.5 µg of DNA)
nuclease-free water	18 µL
10X Buffer Aarl	2 µL
50X oligonucleotide (0.025 mM)	0.6 µL
Aarl	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

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

## ENZYME PROPERTIES

### Enzyme Activity in Thermo Scientific REase Buffers, %

Aarl <sub>+oligo</sub>	B <sub>+oligo</sub>	G <sub>+oligo</sub>	O <sub>+oligo</sub>	R <sub>+oligo</sub>	Tango <sub>+oligo</sub>	2X Tango <sub>+oligo</sub>
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 Aarl (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.

## **PRODUCT USE LIMITATION**

This product is developed, designed and sold exclusively *for research purposes and in vitro use only*. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Please refer to [www.thermoscientific.com/onebio](http://www.thermoscientific.com/onebio) for Material Safety Data Sheet of the product.

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## 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