Thermo SCIENTIFIC

PRODUCT INFORMATION

AloI

#ER1491 100 U

Lot: ___ **Expiry Date: _**

5'... ↓ $_{7}$(N)GAAC(N)$_{6}$TCC(N)$_{(12\text{-}13)}$↓...3'

3'... ↑$_{(12\text{-}13)}$(N)CTTG(N)$_{6}$AGG(N)$_{7}$ ↑...5'

Concentration: 2 U/µL
Supplied with: 1 mL of 10X Buffer R
1 mL of 10X Buffer Tango

Store at -20°C

In total 3 vials. BSA included

www.thermoscientific.com/onebio

RECOMMENDATIONS

1X Buffer R (for 100% AloI digestion)
10 mM Tris-HCl (pH 8.5), 10 mM $MgCl_2$, 100 mM KCl, 0.1 mg/mL BSA.

Incubation temperature
30°C*.

Unit Definition
One unit is defined as the amount of AloI required to digest 1 µg of lambda DNA in 1 hour at 30°C in 50 µL of recommended reaction buffer.

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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* Incubation at 37°C results in 20% activity.

Storage Buffer

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

Recommended Protocol for Digestion

- Add:

nuclease-free water	16 µL
10X Buffer R	2 µL
DNA (0.5-1 µg/µL)	1 µL
AloI	0.5-2 µL**

- Mix gently and spin down for a few seconds.
- Incubate at 30°C for 1-16 hours**.

The digestion reaction may be scaled either up or down.

Recommended Protocol for Digestion of PCR Products Directly after Amplification

- Add:

PCR reaction mixture	10 µL (~0.1-0.5 µg of DNA)
nuclease-free water	18 µL
10X Buffer R	2 µL
AloI	1-2 µL**

- Mix gently and spin down for a few seconds.
- Incubate at 30°C for 1-16 hours**.

** *See* Star Activity.

Thermal Inactivation

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

ENZYME PROPERTIES

Enzyme Activity in Thermo Scientific REase Buffers, %

B	G	O	R	Tango	2X Tango
0-20	0-20	0-20	**100**	20-50	100

Star Activity

An excess of AloI (6 U/µg lambda DNA x 2 hours) may result in star activity.

Methylation Effects on Digestion

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

Stability during Prolonged Incubation

A minimum of 0.1 units of the enzyme is required for complete digestion of 1 µg of lambda DNA in 16 hours at 30°C.

Number of Recognition Sites in DNA

λ	ΦX174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
7	0	0	0	0	1	1

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

Note

- AloI produces double-strand cuts on both sides from interupted recognition site. Its unique feature is a degenerate cleavage point on the 3' side of the recognition sequence (12 or 13 nt away).
- The presence of S-adenosylmethionyne in a reaction mixture results in incomplete cleavage with AloI.
- At least two copies of AloI recognition site are required for efficient cleavage.
- AloI 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 AloI (5 U/µg lambda DNA x 2 hours) (*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 AloI 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

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 for Material Safety Data Sheet of the product.