Thermo SCIENTIFIC

PRODUCT INFORMATION

Alw44I (ApaLI)

#ER0041 1000 U

Lot: ___ **Expiry Date: _**

5'…**G↓T G C A C**…3'
3'…**C A C G T↑G**…5'

Concentration: 10 U/µL
Source: *Acinetobacter lwoffi* RFL44
Supplied with: 1 mL of 10X Buffer Tango

Store at -20°C

In total 2 vials. BSA included

www.thermoscientific.com/onebio

RECOMMENDATIONS

1X Thermo Scientific Tango Buffer (for 100% Alw44I digestion)

33 mM Tris-acetate (pH 7.9), 10 mM magnesium acetate, 66 mM potassium acetate, 0.1 mg/mL BSA.

Incubation temperature

37°C.

Unit Definition

One unit is defined as the amount of Alw44I required to digest 1 µg of lambda DNA-SmaI fragments in 1 hour at 37°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

Tango™ Buffer provided simplifies 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.

Storage Buffer

Alw44I is supplied in: 10 mM Tris-HCl (pH 7.5 at 25°C), 50 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.2 mg/mL BSA and 50% glycerol.

Rev.9

Recommended Protocol for Digestion

- Add:

nuclease-free water	16 µL
10X Buffer Tango	2 µL
DNA (0.5-1 µg/µL)	1 µL
Alw44I	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 Directly after Amplification

- Add:

PCR reaction mixture	10 µL	(~0.1-0.5 µg of DNA)
nuclease-free water	18 µL	
10X Buffer Tango	2 µL	
Alw44I	1-2 µL	

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

Thermal Inactivation

Alw44I 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
50-100	100	0-20	50-100	**100**	50-100

Methylation Effects on Digestion

Dam: never overlaps – no effect.
Dcm: never overlaps – no effect.
CpG: may overlap – blocked.
EcoKI: may overlap – 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 DNA in 16 hours at 37°C.

Digestion of Agarose-embedded DNA

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

Compatible Ends

BfmI

Number of Recognition Sites in DNA

λ	ΦX174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
4	1	3	3	3	2	1*

* According to our experimental data, Alw44I does not cut M13mp18/19 DNA.

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

CERTIFICATE OF ANALYSIS

Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after a 160-fold overdigestion (10 U/µg lambda DNA × 16 hours) with Alw44I.

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 10 units of Alw44I for 4 hours.

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.