Thermo SCIENTIFIC

PRODUCT INFORMATION

AjuI

#ER1951 100 U

Lot: ___ **Expiry Date: _**

5'...↓ $_{7}$(N)G A A(N)$_{7}$ T T G G(N)$_{11}$↓...3'
3'...↑$_{12}$(N)C T T(N)$_{7}$ A A C C(N)$_{6}$ ↑...5'

Concentration:	5 U/µL
Source:	*Acinetobacter junii* RFL46
Supplied with:	1 mL of 10X Buffer R
	1 mL of 10X Buffer Tango
	0.1 mL of 50X SAM Solution (0.5 mM)

Store at -20°C

BSA included

www.thermoscientific.com/onebio

RECOMMENDATIONS

[1X Buffer R]+SAM (for 100% AjuI digestion)
[10 mM Tris-HCl (pH 8.5 at 37°C), 10 mM $MgCl_2$, 100 mM KCl, 0.1 mg/mL BSA] + 0.01 mM S-adenosylmethionine (SAM) (*see* Note).

Incubation Temperature
37°C.

Unit Definition
One unit is defined as the amount of AjuI 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.

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.

Storage Buffer

AjuI 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 (0.5-1µg/µL)	1 µL
50X SAM	0.4 µL
AjuI	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 R	2 µL	
50X SAM	0.6 µL	
AjuI	1-2 µL	

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

Thermal Inactivation

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

ENZYME PROPERTIES

Enzyme Activity in Thermo Scientific REase Buffers, %

B_{+SAM}	G_{+SAM}	O_{+SAM}	R_{+SAM}	$Tango_{+SAM}$	$2X\ Tango_{+SAM}$
0-20	50-100	20-50	**100**	50-100	50-100

Methylation Effects on Digestion

Dam: never overlaps – no effect.
Dcm: never overlaps – no effect.
CpG: may overlap – no effect.
EcoKI: never overlaps – no effect.
EcoBI: never overlaps – no effect.

Stability during Prolonged Incubation

A minimum of 0.5 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
3	1	0	0	0	0	0

Note

- AjuI requires S-adenosylmethionine for activity.
- Complete cleavage of some substrates with AjuI is difficult to achieve.
- AjuI produces double-strand cuts on both sides of the interrupted recognition site. The exact cleavage position depends on the sequences flanking the recognition site and may shift by one base pair. However, for each individual sequence one cleavage position will dominate.

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 with AjuI (10 U/µg lambda DNA x 16 hours).

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 AjuI 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.