# Γhermo SCIENTIFIC

**PRODUCT INFORMATION** 

# Ajil (BmgBl)

#ER1941 200 U

Expiry Date: \_ Lot:

5'...**C A C↓G T C**...3'

3'...G T G↑C A G...5'

Concentration: 5 U/µL Source: Acinetobacter johnsonii RFL47 1 mL of 10X Buffer Ajil Supplied with: 1 mL of 10X Buffer Tango

Store at -20 °C

Unique





**BSA** included www.thermoscientific.com/onebio

# RECOMMENDATIONS

**1X Buffer Ajil** (for 100% Ajil digestion) 10 mM Bis-Tris Propane-HCI (pH 6.5 at 37 °C), 10 mM MgCl<sub>2</sub>, 100 mM KCl and 0.1 mg/mL BSA.

**Incubation Temperature** 

37 °C

## **Unit Definition**

One unit is defined as the amount of Ajil required to digest 1 µg of lambda DNA-Xhol 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 KCI, 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<sup>™</sup> 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**

Ajil 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:

- Add.nuclease-free water16  $\mu$ L10X Buffer Ajil2  $\mu$ LDNA (0.5-1 $\mu$ g/mL)1  $\mu$ LAjil0.5-2  $\mu$ L\*
- 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 $\mu L$ (~0.1-0.5 $\mu g$ of DNA)
nuclease-free water	18 µL
10X Buffer Ajil	2 µL
Ajil	1-2 µL*

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

\* See Overdigestion Assay.

## **Thermal Inactivation**

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

# **ENZYME PROPERTIES**

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

Ajil	В	G	0	R	Tango™	2X Tango™
100	NR	NR	20-50**	NR	NR	20-50**

\*\*Star activity appears at a greater than 5-fold overdigestion (5 U x 1h). NR – buffer is not recommended, because of high star activity.

#### **Methylation Effects on Digestion**

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

## **Stability during Prolonged Incubation**

A minimum of 0.5 units of the enzyme is required for complete digestion of 1  $\mu$ g 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 complete digestion of 1  $\mu$ g of agarose-embedded lambda DNA in 16 hours.

## Number of Recognition Sites in DNA

λ	ФХ174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
17	0	0	0	0	0	0

For CERTIFICATE OF ANALYSIS see back page

## **CERTIFICATE OF ANALYSIS**

#### **Overdigestion Assay**

No detectable change in the specific fragmentation pattern

is observed after a 80-fold overdigestion with Ajil

(5 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 doublestranded labeled oligonucleotides occurred during incubation with 10 units of Ajil 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

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Please refer to <u>www.thermoscientific.com/onebio</u> for Material Safety Data Sheet of the product.

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