

## CERTIFICATE OF ANALYSIS

# AjiI (BtrI)

#ER1941      200u

**Lot:**                      **Expiry Date:**

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

Concentration:      5 units/μl  
Source:                *Acinetobacter johnsonii* RFL47  
Supplied with:      1ml of 10X Buffer Ajil  
                             1ml of 10X Buffer Tango™

**Store at -20°C**



In total 3 vials.

BSA included: Lot# BSA62-313P

## RECOMMENDATIONS

**1X Buffer Ajil** (for 100% Ajil digestion)

10mM Bis-Tris Propane-HCl (pH 6.5 at 37°C),  
10mM MgCl<sub>2</sub>, 100mM KCl and 0.1mg/ml BSA.

**Incubation temperature**

37°C.

**Unit Definition**

One unit is defined as the amount of Ajil required to digest 1μg of lambda DNA-XhoI fragments in 1 hour at 37°C in 50μl of the assay buffer.

**Dilution**

Dilute with the Dilution Buffer (#B19): 10mM Tris-HCl (pH 7.4 at 25°C), 100mM KCl, 1mM EDTA, 1mM DTT, 0.2mg/ml BSA and 50% glycerol.

**Double Digests**

Tango™ Buffer is provided to simplify buffer selection for double digests. 98% of Fermentas Restriction Endonucleases (REases) are active in 1X or 2X concentration of the Tango™ Buffer. Please refer to the Fermentas Catalog or go to [www.fermentas.com/doubledigest](http://www.fermentas.com/doubledigest) to choose the best buffer for REases in your experiments.

Tango™ Buffer:

33mM Tris-acetate (pH 7.9 at 37°C), 10mM magnesium acetate, 66mM potassium acetate, 0.1mg/ml BSA.

**Storage Buffer**

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

## Recommended Protocol for Digestion

- Add:  
Water, nuclease-free (#R0581) 16 $\mu$ l  
10X Buffer Ajil 2 $\mu$ l  
DNA (0.5-1 $\mu$ g/ $\mu$ l) 1 $\mu$ l  
Ajil 0.5-2 $\mu$ 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)  
Water, nuclease-free 18 $\mu$ l  
10X Buffer Ajil 2 $\mu$ l  
Ajil 1-2 $\mu$ l\*
- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours\*.

\* See Note.

## Thermal Inactivation

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

## ENZYME PROPERTIES

### Enzyme Activity in Fermentas REase Buffers, %

Ajil	B	G	O	R	Tango™	2X Tango™
100	NR	NR	20-50**	NR	NR	20-50**

\*\* – Star activity appears at a greater than 5-fold overdigestion (5 units x 1 hour).  
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

Minimum 0.5 units of the enzyme are required for complete digestion of 1 $\mu$ g of lambda DNA in 16 hours at 37°C.

### Digestion of Agarose-embedded DNA

Minimum 5 units of the enzyme are required for complete digestion of 1 $\mu$ g of agarose-embedded lambda DNA in 16 hours.

### Number of Recognition Sites in DNA

$\lambda$	$\Phi$ X174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
17	0	0	0	0	0	0

### Note

Large excess of enzyme (7.5u/ $\mu$ g x 16 hours), low salt concentration or high pH may result in star activity.

For **QUALITY CONTROL ASSAY DATA** see back page

# QUALITY CONTROL ASSAY DATA

## Overdigestion Assay

No detectable change in specific fragmentation pattern is observed after 80-fold overdigestion with Ajil (5u/μg lambda DNA x 16 hours).

## Ligation/Recutting Assay

After 50-fold overdigestion (3u/μg DNA x 17 hours) with Ajil, approximately 80% of DNA fragments can be ligated. No more than 50% of these can be recut due to the asymmetric recognition sequence of Ajil. The remaining uncleaved ligation products may be cut by AatII and Eco72I (PmaCI).

## Labeled Oligonucleotide (LO) Assay

No detectable degradation of single-stranded and double-stranded labeled oligonucleotides was observed after the incubation with 10 units of Ajil for 4 hours.

## Blue/White Cloning Assay

The mixture of pUC57/HindIII, pUC57/PstI and pUC57/Eco32I digests was incubated with 10 units of Ajil for 16 hours. After religation and transformation, 0.2% of white colonies were detected.

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.fermentas.com](http://www.fermentas.com) for Material Safety Data Sheet of the product.