

CERTIFICATE OF ANALYSIS

AloI

#ER1491 100u

Lot: Expiry Date:

5'... ↓ ${}_7(\text{N})\text{GAAC}(\text{N})_6\text{TCC}(\text{N})_{(12-13)}\downarrow\dots3'$
3'... ↑ $_{(12-13)}(\text{N})\text{CTTG}(\text{N})_6\text{AGG}(\text{N})_7\uparrow\dots5'$

Concentration: 2 units/ μl
Source: *E.coli* that carries the cloned *alolR* gene from *Acinetobacter Iwoffi* Ks4-8
Supplied with: 1ml of 10X Buffer R
1ml of 10X Buffer Tango™

Store at -20°C



In total 3 vials.

BSA included: Lot# BSA62-313P

RECOMMENDATIONS

1X Buffer R (for 100% AolI digestion)

10mM Tris-HCl (pH 8.5), 10mM MgCl_2 , 100mM KCl, 0.1mg/ml BSA.

Incubation temperature

30°C^* .

Unit Definition

One unit is defined as the amount of AolI 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): 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 Enzymes are active in a 1X or 2X concentration of Tango™ Buffer. Please refer to the Fermentas Catalog or go to www.fermentas.com/doubledigest to choose the best buffer for your experiments.

1X Tango™ Buffer:

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

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

Storage Buffer

Alol 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:

nuclease-free water	16µl
10X Buffer R	2µl
DNA (0.5-1µg/µl)	1µl
Alol	0.5-2µl**
- 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	10µl (~0.1-0.5µg of DNA)
nuclease-free water	18µl
10X Buffer R	2µl
Alol	1-2µl**
- Mix gently and spin down for a few seconds.
- Incubate at 30°C for 1-16 hours**.

** See Star Activity.

Thermal Inactivation

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

ENZYME PROPERTIES

Enzyme Activity in Fermentas REase Buffers, %

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

Star Activity

An excess of Alol (6u/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 1µg 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

Note

- Alol produces double-strand cuts on both sides from interrupted 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 SAM in a reaction mixture results in incomplete cleavage with Alol.

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

QUALITY CONTROL ASSAY DATA

Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after a 10-fold overdigestion with Alol (5u/μg lambda DNA x 2 hours) (see Star Activity).

Ligation/Recutting Assay

After a 10-fold overdigestion (0.6u/μg DNA x 17 hours) with Alol more than 70% of the digested DNA fragments can be ligated at the 5'-termini concentration of 0.06μM. More than 80% of these sites can be recut.

Labeled Oligonucleotide (LO) Assay

No detectable degradation of single-stranded or double-stranded labeled oligonucleotides occurred during incubation with 10 units of Alol for 4 hours.

Blue/White Cloning Assay

The mixture of pUC57/HindIII, pUC57/PstI and pUC57/Eco32I digests was incubated with 10 units of Alol for 16 hours. After religation and transformation, the background level of white colonies was 0.2%.

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 for Material Safety Data Sheet of the product.