

PRODUCT INFORMATION

AdeI (DraIII)

#ER1231 500 U

Lot: ____ **Expiry Date:** __

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

Concentration: 10 U/μL
Source: *Alcaligenes denitrificans* SS 3-028
Supplied with: 1 mL of 10X Buffer G
 1 mL of 10X Buffer Tango

Store at -20°C



BSA included

www.thermoscientific.com/onebio

RECOMMENDATIONS

1X Buffer G (for 100% Adel digestion)

10 mM Tris-HCl (pH 7.5), 10 mM MgCl₂, 50 mM NaCl,
0.1 mg/mL BSA.

Incubation temperature

37°C.

Unit Definition

One unit is defined as the amount of Adel required to digest 1 μg of lambda DNA 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

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

Adel is supplied in: 10 mM Tris-HCl (pH 7.5 at 25°C), 300 mM KCl, 1 mM DTT, 0.1mM EDTA, 0.2 mg/mL BSA and 50% glycerol.

Recommended Protocol for Digestion

- Add:

nuclease-free water	16 μ L
10X Buffer G	2 μ L
DNA (0.5-1 μ g/ μ L)	1 μ L
Adel	0.5-2 μ 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 μ L (~0.1-0.5 μ g of DNA)
nuclease-free water	18 μ L
10X Buffer G	2 μ L
Adel	1-2 μ L*
- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours*.

* See Star Activity.

Thermal Inactivation

Adel (Dralll) is not inactivated by incubation at 80°C for 20 min.

Inactivation Procedure

- To prepare the digested DNA for electrophoresis:
 - stop the digestion reaction by adding 0.5 M EDTA, pH 8.0 (#R1021), to achieve a 20 mM final concentration. Mix thoroughly, add an electrophoresis loading dye and load onto gel.
- To prepare DNA suitable for further enzymatic reactions:
 - extract with phenol/chloroform, precipitate with ethanol or isopropanol, wash the pellet with 75% cold ethanol and air-dry;
 - dissolve DNA in either nuclease-free water, TE buffer, or a buffer suitable for further applications;
 - check the DNA concentration in the solution.

ENZYME PROPERTIES

Enzyme Activity in Thermo Scientific REase Buffers, %

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

**Star activity appears at a greater than 5-fold overdigestion (5 U x 1h).

Star Activity

An excess of Adel (20 U/ μ g DNA x 1 hour) may result in star activity.

Methylation Effects on Digestion

Dam: never overlaps – no effect.

Dcm: never overlaps – no effect.

CpG: may overlap – cleavage impaired.

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 μ g of DNA in 16 hours at 37°C.

Digestion of Agarose-embedded DNA

A minimum of 10 units of the enzyme is required for complete digestion of 1 μ g of agarose-embedded lambda DNA in 16 hours.

Number of Recognition Sites in DNA

λ	Φ X174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
10	1	0	0	0	1	1

CERTIFICATE OF ANALYSIS

Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after a 15-fold overdigestion with Adel (15 U/ μ g lambda DNA x 1 hour) (*see* Star Activity).

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 Adel 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.

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