Thermo SCIENTIFIC

PRODUCT INFORMATION

Acc65I (Asp718I)

#ER0901 1000 U

Lot: ___ **Expiry Date: _**

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

Concentration:	10 U/µL
Source:	*Acinetobacter aceti* 655
Supplied with:	1 mL of 10X Buffer O
	1 mL of 10X Buffer Tango

Store at -20°C

In total 3 vials. BSA included

www.thermoscientific.com/onebio

RECOMMENDATIONS

1X Buffer O (for 100% Acc65I digestion)
50 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 100 mM NaCl, 0.1 mg/mL BSA.

Incubation temperature
37°C.

Unit Definition
One unit is defined as the amount of Acc65I required to digest 1 µg of lambda DNA-BamHI 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 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

Acc65I is supplied in: 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.

Recommended Protocol for Digestion

- Add:

nuclease-free water	16 µL
10X Buffer O	2 µL
DNA (0.5-1µg/µL)	1 µL
Acc65I	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 O	2 µL
Acc65I	1-2 µL

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

Thermal Inactivation

Acc65I is inactivated by incubation at 65°C for 20 min.

ENZYME PROPERTIES

Enzyme Activity in Thermo Scientific REase Buffers, %

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

Methylation Effects on Digestion

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

Stability during Prolonged Incubation

A minimum 0.3 units of the enzyme is required for complete digestion of 1 µg of lambda DNA in 16 hours at 37°C.

Digestion of Agarose-embedded DNA

A minimum 5 units of the enzyme is required for complete digestion of 1 µg of agarose-embedded lambda DNA in 16 hours.

Compatible Ends

BshNI, Bsp1407I, PfI23II, TatI

Number of Recognition Sites in DNA

λ	ΦX174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
2	0	0	1	1	1	1

Note

Acc65I cleavage is impaired by overlapping *dcm* methylation. To avoid *dcm* methylation, use a *dam*$^-$, *dcm*$^-$ strain such as GM2163 (#M0099).

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 Acc65I (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 Acc65I 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.