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PRODUCT INFORMATION

 Xbal

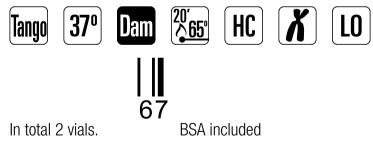
 #ER0681
 1500 U

 Lot:
 Expiry Date:

5'...T \downarrow C T A G A...3' 3'...A G A T C \uparrow T...5'

Concentration:10 U/µLSource:Xanthomonas badriiSupplied with:1 mL of 10X Buffer Tango

Store at -20°C



www.thermoscientific.com/onebio

RECOMMENDATIONS

1X Thermo Scientific Tango Buffer (for 100% Xbal

digestion)

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

Incubation temperature

37°C.

Unit Definition

One unit is defined as the amount of Xbal required to digest 1 μ g of lambda DNA *dam*⁻-Smal fragments in 1 hour at 37°C in 50 μ L 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

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 <u>www.thermoscientific.com/doubledigest</u> to choose the best buffer for your experiments.

Storage Buffer

Xbal 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.

Rev.13

Recommended Protocol for Digestion

- Add:
 - Nuclease-free water16 μ L10X Buffer Tango2 μ LDNA (0.5-1 μ g/ μ L)1 μ LXbal0.5-2 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:

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

 This volume of the enzyme is recommended for preparations of standard concentrations (10 U/µL), whereas HC enzymes (50 U/µL) should be diluted with Dilution Buffer to obtain 10 U/µL concentration.

Thermal Inactivation

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

ENZYME PROPERTIES

Enzyme Activity in Thermo Scientific REase Buffers, %

В	G	0	R	Tango	2X Tango
50-100	50-100	20-50	0-20	100	50-100

Methylation Effects on Digestion

Dam: may overlap – blocked. Dcm: never overlaps – no effect. CpG: never overlaps – no effect. 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 1 μ 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 μ g of agarose-embedded lambda DNA in 16 hours.

Compatible Ends

Bcul, Eco130l, Nhel, XmaJl

Number of Recognition Sites in DNA

λ	Ф Х174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
1	0	0	1	1	1	1

Note

Xbal is blocked by overlapping *dam* methylation. To avoid *dam* methylation, use a *dam*⁻, *dcm*⁻ strain such as GM2163 (#M0099).

CERTIFICATE OF ANALYSIS

Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after a 160-fold overdigestion with Xbal (10 U/µg lambda DNA $dam^- \times 16$ 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 occured during incubation with 10 units of Xbal 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 <u>www.thermoscientific.com/onebio</u> for Material Safety Data Sheet of the product.

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