Thermo s c i e n t i f i c

PRODUCT INFORMATION

Eco130I (Styl) **#ER0411** 2500 U

Lot: ____ Expiry Date: _

5'...**C↓C W W G G**...3'

3'....**G G W W C^C...**5'

Concentration:	10 U/μL
Source:	<i>Escherichia coli</i> RFL130
Supplied with:	1 mL of 10X Buffer 0
	1 mL of 10X Buffer Tango

Store at -20°C 0 37° 20° 7° 10° 10°

IBSA included

RECOMMENDATIONS

1X Buffer 0 (for 100% Eco130I digestion)

50 mM Tris-HCl (pH 7.5), 10 mM MgCl₂, 100 mM NaCl, 0.1 mg/mL BSA.

Incubation temperature

37°C.

Unit Definition

One unit is defined as the amount of Eco130I required to digest 1 μ g of lambda DNA 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

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 <u>www.thermoscientific.com/doubledigest</u> 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.

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Rev.10

Storage Buffer

Eco130I is supplied in: 10 mM Tris-HCI (pH 7.5 at 25°C), 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.2 mg/mL BSA and 50% glycerol.

Recommended Protocol for Digestion

• Add:

nuclease-free water	16 µL		
10X Buffer 0	2 µL		
DNA (0.5-1 μg/μL)	1 µL		
Eco130I	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 Eco1301 1-2 µL

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

Thermal Inactivation

Eco130I is inactivated by incubation at 65°C for 20 min.

ENZYME PROPERTIES

Enzyme Activity in Thermo Scientific REase Buffers, %

B	G	0	R	Tango	2X Tango	
0-20	20-50	100	50-100	50-100	100	_

Methylation Effects on Digestion

Dam: never overlaps - no effect. 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 0.2 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 of 5 units of the enzyme is required for complete digestion of 1 µg of agarose-embedded lambda DNA in 16 hours.

Compatible Ends

C↓CTAGG – Bcul, Nhel, Xbal, XmaJI

 $C\downarrow CATGG - AfIIII, PscI, FatI, PagI, BtgI, NcoI.$

Number of Recognition Sites in DNA

λ	ФХ174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
10	0	1	0	0	0	0

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 Eco130I (10 $u/\mu g$ 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 doublestranded labeled oligonucleotides occurred during incubation with 10 units of Eco130I 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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