

PRODUCT INFORMATION

Cfr42I (SaclI)

#ER0201 1200 U

Lot: ____ **Expiry Date:** __

5'... C C G C↓G G...3'
3'... G G↑C G C C...5'

Concentration: 10 U/μL

Source: *E.coli* that carries the cloned *cfr42IR* gene from *Citrobacter freundii* RFL42

Supplied with: 1 mL of 10X Buffer B
1 mL of 10X Buffer Tango

Store at -20°C



In total 3 vials.

BSA included

RECOMMENDATIONS

1X Buffer B (for 100% Cfr42I digestion)

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

Incubation temperature

37°C.

Unit Definition

One unit is defined as the amount of Cfr42I 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

Cfr42I is supplied in: 10 mM potassium phosphate (pH 7.4 at 25°C), 100 mM NaCl, 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 B	2 µL
DNA (0.5-1 µg/µL)	1 µL
Cfr42I	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 B	2 µL
Cfr42I	1-2 µL
- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours.

Thermal Inactivation

Cfr42I 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
100	50-100	0-20	0-20	50-100	0-20

Methylation Effects on Digestion

Dam: never overlaps – no effect.
Dcm: never overlaps – no effect.
CpG: completely overlaps – blocked.
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 µg of lambda DNA in 16 hours at 37°C.

Digestion of Agarose-embedded DNA

A minimum of 20 units of the enzyme is required for complete digestion of 1 µg of agarose-embedded lambda DNA in 16 hours.

Compatible Ends

Bsh1285I

Number of Recognition Sites in DNA

λ	ΦX174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
4	1	0	0	0	0	0

For **CERTIFICATE OF ANALYSIS** see back page

Note

- Particular sites in λ and Φ X174 DNAs are difficult to cleave with Cfr42I, as well as with its prototype SacII. At least two copies of Cfr42I recognition site are required for efficient cleavage.
- Cfr42I cleavage is affected by high salt concentration. Trace amounts of sodium chloride remaining in the substrate DNA after completion of upstream applications may inhibit enzyme activity and result in impaired DNA cleavage.

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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CERTIFICATE OF ANALYSIS

Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after a 160-fold overdigestion with Cfr42I (10 U/ μ g lambda DNA \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 double-stranded labeled oligonucleotides occurred during incubation with 10 units of Cfr42I 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