

CERTIFICATE OF ANALYSIS

CfrI (EaeI)

#ER0162 1000 u

Lot: Expiry Date:

5'...Py↓G G C C Pu...3'

3'...Pu C C G G↑Py...5'

Concentration: 10 u/μl

Source: *E.coli* that carries the cloned *cfrI*R gene from *Citrobacter freundii* RFL1

Supplied with: 1 ml of 10X Buffer Tango™

Store at -20°C



In total 2 vials.

BSA included: Lot# BSA62-313P

RECOMMENDATIONS

1X Buffer Tango™ (for 100% CfrI 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 CfrI required to digest 1 μg of lambda DNA *dcm*⁻ 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

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 www.fermentas.com/doubledigest to choose the best buffer for your experiments.

Storage Buffer

CfrI is supplied in: 10 mM Tris-HCl (pH 8.5 at 25°C), 500 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.5 mg/ml BSA and 50% glycerol.

Recommended Protocol for Digestion

(customers can modify this protocol according to their needs)

- Add:

nuclease-free water	16 μ l
10X Buffer Tango™	2 μ l
DNA (0.5-1 μ g/ μ l)	1 μ l
CfrI	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 Tango™	2 μ l
CfrI	1-2 μ l
- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours.

Thermal Inactivation

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

ENZYME PROPERTIES

Enzyme Activity in Fermentas REase Buffers, %

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

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

Methylation Effects on Digestion

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

Stability during Prolonged Incubation

A minimum of 1 unit 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

Bsp120I, Eco52I, NotI

Number of Recognition Sites in DNA

λ	Φ X174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
39	2	6	3	3	3	3

For **QUALITY CONTROL ASSAY DATA** see back page

QUALITY CONTROL ASSAY DATA

Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after a 160-fold overdigestion with CfrI (10 u/μg lambda DNA *dcm*⁻ x 16 hours).

Ligation/Recutting Assay

After a 50-fold overdigestion (3 u/μg DNA x 17 hours) with CfrI, more than 95% of the digested DNA fragments can be ligated at a 5'-termini concentration of 0.4 μM. More than 95% of these sites can be recut.

Labeled Oligonucleotide (LO) Assay

No detectable degradation of single-stranded or double-stranded labeled oligonucleotides occurred during incubation with 10 units of CfrI for 4 hours.

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.fermentas.com for Material Safety Data Sheet of the product.