

CERTIFICATE OF ANALYSIS

# CfrI (EaeI)

#ER0161      200 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*<sup>-</sup> 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](http://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 $\mu$ l
10X Buffer Tango™	2 $\mu$ l
DNA (0.5-1 $\mu$ g/ $\mu$ l)	1 $\mu$ l
CfrI	0.5-2 $\mu$ 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 $\mu$ l (~0.1-0.5 $\mu$ g of DNA)
nuclease-free water	18 $\mu$ l
10X Buffer Tango™	2 $\mu$ l
CfrI	1-2 $\mu$ 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  $\mu$ 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  $\mu$ g of agarose-embedded lambda DNA in 16 hours.

### Compatible Ends

Bsp120I, Eco52I, NotI

### Number of Recognition Sites in DNA

$\lambda$	$\Phi$ 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*<sup>-</sup> 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](http://www.fermentas.com) for Material Safety Data Sheet of the product.