Fermentas
LIFE SCIENCES

CERTIFICATE OF ANALYSIS

Cfr9I (XmaI)

#ER0171 300 u

Lot: **Expiry Date:**

5'…**C↓C C G G G**…3'
3'…**G G G C C↑C**…5'

Concentration: 10 u/µl
Source: *E.coli* that carries the cloned *cfr9IR* gene from *Citrobacter freundii* RFL9
Supplied with: 1 ml of 10X Buffer Cfr9I
1 ml of 10X Buffer Tango™

Store at -20°C

In total 3 vials.

BSA included: Lot# BSA62-313P

ISO 9001 ISO 14001
www.fermentas.com

RECOMMENDATIONS

1X Buffer Cfr9I (for 100% Cfr9I digestion)
10 mM Tris-HCl (pH 7.2), 5 mM $MgCl_2$, 200 mM sodium glutamate, 0.1 mg/ml BSA.

Incubation temperature
37°C.

Unit Definition
One unit is defined as the amount of Cfr9I required to digest 1 µg of lambda DNA-HindIII fragments in 1 hour at 37°C in 50 µl of recommended reaction buffer (containing 2µg DNA fragments).

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.
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

Cfr9I is supplied in: 10 mM Tris-HCl (pH 7.5 at 25°C), 250 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 Cfr9I	2 µl
DNA (0.5-1 µg/µl)	1 µl**
Cfr9I	1-2 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 Cfr9I	2 µl
Cfr9I	1-2 µl

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

**See* Note.

Thermal Inactivation

Cfr9I is inactivated by incubation at 65°C for 20 min.

ENZYME PROPERTIES

Enzyme Activity in Fermentas REase Buffers, %

Cfr9I	B	G	O	R	Tango™	2X Tango™
100	0-20	0-20	0-20	0-20	20-50	0-20

Methylation Effects on Digestion

Dam: never overlaps – no effect.
Dcm: never overlaps – no effect.
CpG: completely overlaps – cleavage impaired.
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

BshTI, BsaWI, Cfr10I, Eco88I, Kpn2I, NgoMIV, SgrAI.

Number of Recognition Sites in DNA

λ	ΦX174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
3	0	0	1	1	1	1

Note

To achieve complete digestion of substrate with Cfr9I, the concentration of DNA in reaction buffer should not be less than 50 µg/ml.

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 Cfr 9I (10 u/µg lambda DNA x 16 hours).

Ligation/Recutting Assay

After a 50-fold overdigestion (3 u/µg DNA x 17 hours) with Cfr 9I, more than 95% of the digested DNA fragments can be ligated at a 5'-termini concentration of 0.03 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 Cfr 9I 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.

(6) Revised 24.08.2006