Thermo SCIENTIFIC

PRODUCT INFORMATION

Cfr10I (BsrFI)

#ER0181 200 U

Lot: ___ **Expiry Date: _**

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

Concentration:	10 U/µL
Source:	*E.coli* that carries the cloned *cfr10IR* gene from *Citrobacter freundii* RFL10
Supplied with:	1 mL of 10X Buffer Cfr10I 1 mL of 10X Buffer Tango™

Store at -20°C

BSA included

www.thermoscientific.com/onebio

RECOMMENDATIONS

1X Buffer Cfr10I (for 100% Cfr10I digestion)
10 mM Tris-HCl (pH 8.0), 5 mM $MgCl_2$, 100 mM NaCl, 0.02% Triton X-100, 1 mg/mL BSA.

Incubation temperature
37°C.

Unit Definition
One unit is defined as the amount of Cfr10I 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.

Rev.11

Storage Buffer

Cfr10I is supplied in: 10 mM potassium phosphate (pH 7.4 at 25°C), 100 mM KCl, 1 mM DTT, 1 mM EDTA, 0.2 mg/mL BSA and 50% glycerol.

Recommended Protocol for Digestion

- Add:

nuclease-free water	16 µL
10X Buffer Cfr10I	2 µL
DNA (0.5-1 µg/µL)	1 µL
Cfr10I	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 Cfr10I	2 µL
Cfr10I	1-2 µL*****

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

***** *See* Star Activity on back page.

Thermal Inactivation

Cfr10I is not inactivated by incubation at 80°C for 20 min.

Inactivation Procedure

- To prepare the digested DNA for electrophoresis:
  – stop the digestion reaction by adding 0.5 M EDTA, pH 8.0 (#R1021), to achieve a 20 mM final concentration. Mix thoroughly, add an electrophoresis loading dye and load onto gel.
- To prepare DNA suitable for further enzymatic reactions:
  – extract with phenol/chloroform, precipitate with ethanol or isopropanol, wash the pellet with 75% cold ethanol and air-dry;
  – dissolve DNA in either nuclease-free water, TE buffer, or a buffer suitable for further applications;
  – check the DNA concentration in the solution.

For **ENZYME PROPERTIES** and **CERTIFICATE OF ANALYSIS** *see* back page

ENZYME PROPERTIES

Enzyme Activity in Thermo Scientific REase Buffers, %

Cfr10I	B	G	O	R	Tango	2X Tango
100**	0-20	20-50	20-50	50-100**	20-50	50-100

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

Star Activity

An excess of Cfr10I (10 U/µg DNA × 1 hour) or low salt concentration may result in star activity.

Methylation Effects on Digestion

Dam: never overlaps – no effect.
Dcm: never overlaps – no effect.
CpG: completely overlaps – blocked.
EcoKI: may overlap – no effect.
EcoBI: may overlap – 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 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, Cfr9I, Eco88I, Kpn2I, MreI, NgoMIV, SgrAI

Number of Recognition Sites in DNA

λ	ΦX174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
61	0	7	1	1	2	1

Note

For cleavage with Cfr10I at least two copies of its recognition sequence are required.

CERTIFICATE OF ANALYSIS

Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after a 5-fold overdigestion with Cfr10I (5 U/µg lambda DNA × 1 hour) (*see* Star Activity).

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 Cfr10I for 4 hours.

Quality authorized by:  Jurgita Zilinskiene