Thermo SCIENTIFIC

**PRODUCT INFORMATION**

# **Cfr42I** (SacII)

**#ER0205** 2000 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

**www.thermoscientific.com/onebio**

## RECOMMENDATIONS

**1X Buffer B** (for 100% Cfr42I digestion)
10 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 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 (pH7.4 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.12

## 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 ΦX174 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.



# CERTIFICATE OF ANALYSIS

## Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after a 160-fold overdigestion with Cfr42I (10 U/µg lambda DNA × 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