

#### **PRODUCT INFORMATION**

## CpoI (RsrII)

**#ER0742** 1000 U

Lot: \_\_\_ Expiry Date: \_

5'...C G↓G W C C G...3' 3'...G C C W G↑G C...5'

Concentration: 10 U/µL

Source: Caseobacter polymorphus
Supplied with: 1 mL of 10X Buffer Tango

Store at -20°C











BSA included

www.thermoscientific.com/onebio

#### RECOMMENDATIONS

**1X Thermo Scientific Tango Buffer** (for 100% Cpol 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 Cpol required to digest  $1\mu g$  of lambda DNA in 1 hour at  $37^{\circ}C$  in  $50 \mu L$  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<sup>™</sup> Buffer provided simplifies buffer selection for double digests. 98% of Thermo Scientific restriction enzymes are active in a 1X or 2X concentration of Tango Buffer. Please go to <a href="https://www.thermoscientific.com/doubledigest">www.thermoscientific.com/doubledigest</a> to choose the best buffer for your experiments.

#### **Storage Buffer**

Cpol is supplied in: 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.



## **Recommended Protocol for Digestion**

Add:

nuclease-free water  $16~\mu L$  10X~Buffer~Tango  $2~\mu L$   $DNA~(0.5-1~\mu g/\mu L)$   $1~\mu L$  Cpol  $0.5-2~\mu L$ 

- 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 Cpol 1-2  $\mu$ L

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

#### **Thermal Inactivation**

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

#### **ENZYME PROPERTIES**

## **Enzyme Activity in Thermo Scientific REase Buffers, %**

В	G	0	R	Tango	2X Tango
20-50	50-100	50-100	20-50	100	50-100

#### **Methylation Effect 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 0.5 units of the enzyme is required for complete digestion of 1  $\mu$ g of lambda DNA in 16 hours at 37°C.

## **Compatible Ends**

Cfr 13I, Eco 47I, Psp 5II, San DI

#### **Number of Recognition Sites in DNA**

λ	ФХ174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
5	0	0	0	0	0	0

For **CERTIFICATE OF ANALYSIS** see back page

#### **CERTIFICATE OF ANALYSIS**

#### **Overdigestion Assay**

No detectable change in the specific fragmentation pattern is observed after a 160-fold overdigestion with Cpol (10 U/µg lambda DNA x 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 doublestranded labeled oligonucleotides occurred during incubation with 10 units of CpoI 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

#### 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.thermoscientific.com/onebio for Material Safety Data Sheet of the product.

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