

## PRODUCT INFORMATION

# CseI (HgaI)

**#ER1901** 100 U

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

5'... **G A C G C(N)<sub>5</sub>** ↓...3'

3'... **C T G C G(N)<sub>10</sub>** ↑...5'

Concentration: 5 U/μL

Source: *E.coli* that carries the cloned *cseI/R* gene  
from *Chryseobacterium species* RFL1

Supplied with: 1 mL of 10X Buffer R  
1 mL of 10X Buffer Tango

**Store at -20°C**



BSA included

## RECOMMENDATIONS

**1X Buffer R** (for 100% CseI digestion)

10 mM Tris-HCl (pH 8.5), 10 mM MgCl<sub>2</sub>, 100 mM KCl,  
0.1 mg/mL BSA.

**Incubation temperature**

37°C.

**Unit Definition**

One unit is defined as the amount of CseI required to digest 1 μg of pBR322 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](http://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.

## Storage Buffer

Csel is supplied in: 10 mM Tris-HCl (pH 7.5 at 25°C), 100 mM KCl, 1 mM DTT, 0.1 mM EDTA and 50% glycerol.

## Recommended Protocol for Digestion

- Add:

nuclease-free water	16 µL
10X Buffer R	2 µL
DNA (0.5-1 µg/µL)	1 µL
Csel	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 R	2 µL
Csel	1-2 µL*
- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours\*.

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\* See Overdigestion Assay on back page.

## Thermal Inactivation

Only small amounts of Csel (up to 10 units) can be inactivated 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, %

B	G	O	R	Tango	2X Tango
NR	50-100**	50-100	<b>100</b>	100**	50-100

\*\*Star activity appears at a greater than 5-fold overdigestion (5 u x 1h).  
NR – buffer is not recommended, because of high star activity.

### Methylation Effects on Digestion

Dam: never overlaps – no effect.

Dcm: never overlaps – no effect.

CpG: completely overlaps – blocked.

EcoKI: never overlaps – no effect.

EcoBI: may overlap – effect not determined.

### Stability during Prolonged Incubation

A minimum of 0.5 units of the enzyme is required for complete digestion of 1 µg of pBR322 DNA in 16 hours at 37°C.

### Number of Recognition Sites in DNA

λ	ΦX174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
102	14	11	4	4	4	7

### Note

- For cleavage with Csel at least two copies of its recognition sequence are required.
- Csel may remain associated with the cleaved DNA. This may cause DNA band shifting during electrophoresis. To avoid atypical DNA band patterns, use the 6X DNA Loading Dye&SDS Solution (R1151) for sample preparation or heat the digested DNA in the presence of SDS prior to electrophoresis.

## CERTIFICATE OF ANALYSIS

### Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after a 80-fold overdigestion with Csel (5 U/µg pBR322 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 double-stranded labeled oligonucleotides occurred during incubation with 10 units of Csel 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.thermoscientific.com/onebio](http://www.thermoscientific.com/onebio) for Material Safety Data Sheet of the product.

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