

CERTIFICATE OF ANALYSIS

# Lsp1109I (BbvI)

#ER2071      200 u

Lot:                      Expiry Date:

5'...G C A G C(N)<sub>8</sub> ↓...3'

3'...C G T C G(N)<sub>12</sub>↑...5'

Concentration:      3 u/μl

Source:                *E.coli* that carries cloned *Lsp1109IR*  
gene from *Listeria species* RFL1109

Supplied with:      1 ml of 10X Buffer Lsp1109I  
1 ml of 10X Buffer Tango™

Store at -20°C



In total 3 vials.

BSA included: Lot# BSA62-313P

## RECOMMENDATIONS

**1X Buffer Lsp1109I** (for 100% Lsp1109I digestion)  
10 mM Tris-HCl (pH 8.0), 5mM MgCl<sub>2</sub>, 100 mM KCl,  
0.02% Triton X-100, 1 mM 2-mercaptoethanol and  
0.1 mg/ml BSA.

**Incubation temperature**  
37°C.

### Unit Definition

One unit is defined as the amount of Lsp1109I 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

Please refer to the Fermentas Catalog or go to [www.fermentas.com/doubledigest](http://www.fermentas.com/doubledigest) to choose the best buffer for your experiments.

### Storage Buffer

Lsp1109I is supplied in: 10 mM Tris-HCl (pH 7.4 at 25°C), 100 mM KCl, 1 mM DTT, 1mM EDTA, 0.2 mg/ml BSA and 50% glycerol.

## Recommended Protocol for Digestion

- Add:
  - nuclease-free water 16  $\mu$ l
  - 10X Buffer Lsp1109I 2  $\mu$ l
  - DNA (0.5-1  $\mu$ g/ $\mu$ l) 1  $\mu$ l
  - Lsp1109I 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 Lsp1109I 2  $\mu$ l
  - Lsp1109I 1-2  $\mu$ l\*
- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours\*.

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\* See Star Activity.

## Thermal Inactivation

Lsp1109I is inactivated by incubation at 65°C for 20 min.

## ENZYME PROPERTIES

### Enzyme Activity in Fermentas REase Buffers, %

Lsp1109I	B	G	O	R	Tango™	2X Tango™
100	0-20	20-50	50-100**	100	20-50**	20-50**

\*\*Star activity appears at a greater than 5-fold overdigestion (5 u x 1 h).

### Star Activity

A large excess of the enzyme (4 u/ $\mu$ g DNA x 16 hours) may result in star activity.

### Methylation Effects on Digestion

Dam: never overlaps – no effect.

Dcm: never overlaps – no effect.

CpG: may overlap – no effect.

EcoKI: never overlaps – no effect.

EcoBI: may overlap – effect not determined.

### Stability during Prolonged Incubation

A minimum of 0.3 units of the enzyme is required for complete digestion of 1  $\mu$ g of pBR322 in 16 hours at 37°C.

### Number of Recognition Sites in DNA

$\lambda$	$\Phi$ X174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
199	14	21	12	12	12	10

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 32-fold overdigestion with Lsp1109I (2 u/μg pBR322 DNA x 16 hours) (see Star Activity).

## Ligation/Recutting Assay

After a 10-fold overdigestion (0.6 u/μg DNA x 17 hours) with Lsp1109I, more than 95% of the digested DNA fragments can be ligated at a 5'-termini concentration of 0.98 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 Lsp1109I for 4 hours.

Quality authorized by:



Jurgita Zilinskiene

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## **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](http://www.fermentas.com) for Material Safety Data Sheet of the product.