

CERTIFICATE OF ANALYSIS

# HindIII

#ER0503 HC, 25000 u

Lot: Expiry Date:

5'...**A↓A G C T T**...3'  
3'...**T T C G A↑A**...5'

Concentration: 50 u/μl  
Source: *Haemophilus influenzae* Rd  
Supplied with: 2x1 ml of 10X Buffer R  
1 ml of 10X Buffer Tango™

Store at -20°C



In total 4 vials.

BSA included: Lot# BSA62-313P

## RECOMMENDATIONS

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

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

**Incubate at 37°C.**

### Unit Definition

One unit is defined as the amount of HindIII required to digest 1 μg 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

Tango™ Buffer is provided to simplify buffer selection for double digests. 98% of Fermentas Restriction Enzymes are active in a 1X or 2X concentration of Tango™ Buffer. 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.

1X Tango™ Buffer:

33 mM Tris-acetate (pH 7.9), 10 mM magnesium acetate, 66 mM potassium acetate, 0.1 mg/ml BSA.

### Storage Buffer

HindIII is supplied in: 10 mM Tris-HCl (pH 7.5 at 25°C), 250 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.2 mg/ml BSA and 50% glycerol.

## Recommended Protocol for Digestion

- Add:

nuclease-free water	16 $\mu$ l
10X Buffer R	2 $\mu$ l
DNA (0.5-1 $\mu$ g/ $\mu$ l)	1 $\mu$ l
HindIII	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 R	2 $\mu$ l
HindIII	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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\* This volume of the enzyme is recommended for preparations of standard concentrations (10 u/ $\mu$ l), whereas HC enzymes (50 u/ $\mu$ l) should be diluted with Dilution Buffer to obtain 10u/ $\mu$ l concentration.

## Thermal Inactivation

HindIII is inactivated by incubation at 80°C for 20 min.

## ENZYME PROPERTIES

### Enzyme Activity in Fermentas REase Buffers, %

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

### Methylation Effects on Digestion

Dam: never overlaps – no effect.  
Dcm: never overlaps – no effect.  
CpG: never overlaps – no effect.  
EcoKI: never overlaps – no effect.  
EcoBI: may overlap – cleavage impaired.

### Stability during Prolonged Incubation

A minimum of 0.1 units of the enzyme is required for complete digestion of 1  $\mu$ g of lambda DNA in 16 hours at 37°C.

### Digestion of Agarose-embedded DNA

A minimum 5 units of the enzyme is required for complete digestion of 1  $\mu$ g of agarose-embedded lambda DNA in 16 hours.

### Number of Recognition Sites in DNA

$\lambda$	$\Phi$ X174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
7	0	1	1	1	1	1

# QUALITY CONTROL ASSAY DATA

## Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after a 160-fold overdigestion with HindIII ( 10 u/μg lambda DNA x 16 hours).

## Ligation/Recutting Assay

After a 50-fold overdigestion (3 u/μg DNA x 17 hours) with HindIII, more than 95% of the digested DNA fragments can be ligated at a 5'-termini concentration of 0.07 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 HindIII for 4 hours.

## Blue/White Cloning Assay

pUC57 was incubated with 10 units of HindIII for 16 hours. After religation and transformation, the background level of white colonies was 0.2%.

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