

CERTIFICATE OF ANALYSIS

HphI

#ER1102 1500 u

Lot: Expiry Date:

5'... G G T G A (N)₈ ↓...3'
3'... C C A C T (N)₇ ↑...5'

Concentration: 10 u/μl
Source: *E.coli* that carries the cloned *hphIR* gene from *Haemophilus parahaemolyticus*
Supplied with: 1 ml of 10X Buffer B
1 ml of 10X Buffer Tango™

Store at -20°C



In total 3 vials.

BSA included: Lot# BSA62-313P

RECOMMENDATIONS

1X Buffer B (for 100% HphI digestion)

10 mM Tris-HCl (pH 7.5), 10 mM MgCl₂, 0.1 mg/ml BSA.

Incubation temperature

37°C.

Unit Definition

One unit is defined as the amount of HphI required to digest 1 μg of lambda DNA *dam*⁻ 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 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

HphI is supplied in: 10 mM Tris-HCl (pH 7.5 at 25°C), 50 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 μ l
10X Buffer B	2 μ l
DNA (0.5-1 μ g/ μ l)	1 μ l
HphI	0.5-2 μ 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 μ l (~0.1-0.5 μ g of DNA)
nuclease-free water	18 μ l
10X Buffer B	2 μ l
HphI	1-2 μ l*
- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours*.

* See Note.

Thermal Inactivation

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

ENZYME PROPERTIES

Enzyme Activity in Fermentas REase Buffers, %

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

Methylation Effects on Digestion

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

Stability during Prolonged Incubation

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

Number of Recognition Sites in DNA

λ	Φ X174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
168	9	12	7	7	6	18

Note

A large excess of HphI (10 u/ μ g DNA x 16 hours) may result in star activity.

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 80-fold overdigestion with HphI (5 u/μg lambda DNA x 16 hours) (see Star Activity).

Ligation/Recutting Assay

After a 50-fold overdigestion (3 u/μg DNA x 17 hours) with HphI, more than 70% of the digested DNA fragments can be ligated at a 5'-termini concentration of 1.7 μM. More than 90% of these sites can be recut.

Quality authorized by:



Jurgita Zilinskiene

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Certain countries are out of the scope of patent coverage.

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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 for Material Safety Data Sheet of the product.