Thermo SCIENTIFIC

PRODUCT INFORMATION

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

BSA included

www.thermoscientific.com/onebio

RECOMMENDATIONS

1X Buffer B (for 100% HphI digestion)

10 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 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 (pH7.4 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 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

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 µL
10X Buffer B	2 µL
DNA (0.5-1 µg/µL)	1 µL
HphI	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 B	2 µL
HphI	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.

Thermal Inactivation

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

ENZYME PROPERTIES

Enzyme Activity in Thermo Scientific 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 µg of lambda DNA in 16 hours at 37°C.

Number of Recognition Sites in DNA

λ	ΦX174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
168	9	12	7	7	6	18

Note

HphI is blocked by overlapping *dam* methylation. To avoid *dam* methylation, use a *dam*$^-$, *dcm*$^-$ strain such as GM2163 (#M0099).

For **CERTIFICATE OF ANALYSIS** *see* back page

CERTIFICATE OF ANALYSIS

Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after a 80-fold overdigestion with HphI (5 U/µg lambda DNA *dam*$^{-}$ 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.

Quality authorized by:  Jurgita Zilinskiene