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PRODUCT INFORMATION

 Hincll (Hindll)

 #ER0491
 500 U

 Lot:

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

Concentration:10 U/µLSupplied with:1 mL of 10X Buffer Tango

Store at -20°C



BSA included

www.thermoscientific.com/onebio

RECOMMENDATIONS

1X Thermo Scientific Tango Buffer (for 100% Hincll

digestion)

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

Incubation temperature

37°C.

Unit Definition

One unit is defined as the amount of Hincll required to digest 1 μg of 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 provided simplifies buffer selection for double digests. 98% of Thermo Scientific restriction enzymes are active in a 1X or 2X concentration of Tango Buffer. Please go to

www.thermoscientific.com/doubledigest

to choose the best buffer for your experiments.

Storage Buffer

Hincll is supplied in: 10 mM Tris-HCl (pH 7.5 at 25°C), 200 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.5 mg/mL BSA and 50% glycerol.

Rev.10

Recommended Protocol for Digestion

• Add:

| / 1001 | |
|---------------------|----------|
| nuclease-free water | 16 µL |
| 10X Buffer Tango | 2 µL |
| DNA (0.5-1 μg/μL) | 1 µL |
| Hincll | 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:

 $\begin{array}{ll} \mbox{PCR reaction mixture} & 10 \ \mbox{μL$} \ (\sim 0.1 \ -0.5 \ \mbox{μg of DNA$}) \\ \mbox{nuclease-free water} & 18 \ \mbox{μL$} \\ \mbox{10X Buffer Tango} & 2 \ \mbox{μL$} \\ \mbox{Hincll} & 1 \ \ 2 \ \mbox{μL$} \end{array}$

- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours.

Thermal Inactivation

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

ENZYME PROPERTIES

Enzyme Activity in Thermo Scientific REase Buffers, %

| В | G | 0 | R | Tango | 2X Tango |
|--------|--------|-------|--------|-------|----------|
| 50-100 | 50-100 | 20-50 | 50-100 | 100 | 50-100 |

Methylation Effects on Digestion

Dam: never overlaps – no effect. Dcm: never overlaps – no effect. CpG: may overlap – cleavage impaired. EcoKI: may overlap – blocked. 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 | Ф Х174 | pBR322 | pUC57 | pUC18/19 | pTZ19R/U | M13mp18/19 |
|----|---------------|--------|-------|----------|----------|------------|
| 35 | 13 | 2 | 1 | 1 | 1 | 1 |

For CERTIFICATE OF ANALYSIS see back page

CERTIFICATE OF ANALYSIS

Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after a 160-fold overdigestion with Hincll (10 U/ μ g lambda DNA \times 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 doublestranded labeled oligonucleotides occurred during incubation with 10 units of HincII 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 <u>www.thermoscientific.com/onebio</u> for Material Safety Data Sheet of the product.

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