



GeneSpin Srl Via Friuli, 51 -20135 Milano  
 P.IVA e C.F. 04520270960  
 administration@pec.genespin.com  
 info@genespin.com

## DATA SHEET

info@genespin.com - www.genespin.com

### HindIII

cat. no	Units
STS -HindIII	7500 Units
	5 x 7500 Units

**Unit Definition:** One unit is the amount of enzyme required to completely digest 1 µg of Lambda DNA (7 sites) in 1 hour in a total reaction volume of 50 µl. Enzyme activity was determined in the recommended reaction buffer.

For general laboratory use.

**Shipping:** shipped on gel packs

**Storage Conditions:** store at -20 °C

**Additional Storage Conditions:** avoid freeze/thaw cycles

**Shelf Life:** 12 months

**Form:** liquid (Supplied in 10 mM Tris-HCl pH 7.4, 50 mM KCl, 0.1 mM EDTA, 1 mM DTT, 500 µg/ml BSA and 50 % [v/v] glycerol)

**Concentration:** 10 units/µl

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5'... A ↓ A G C T T ...3'
3'... T T C G A ↑ A ...5'
  
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**Source:** Haemophilus influenzae Rd

**Supplied with:** 10x Universal Buffer (UB)

Recommended 50 µl assay

5 µl 10x Universal Buffer (UB)

1 µg pure DNA<sup>1</sup> or PCR product<sup>2</sup>

10 units enzyme

fill up to 50 µl PCR grade water

Optimum buffer condition for restriction is 1x UB

<sup>1</sup> Supercoiled or high molecular weight DNA (e.g. plant genomic DNA) may require longer incubation time or higher amount of enzyme.

<sup>2</sup> Some enzymes may require additional DNA bases flanking the restriction site for complete digestion.

#### Protocol:

- The enzyme should not exceed 10 % of total reaction volume.
- Add enzyme as last component. Mix components well before adding enzyme. After enzyme addition, mix gently by pipetting. Do not vortex.
- Incubate 5 to 10 min. at 60 °C.
- Stop reaction by alternatively:
  - Addition of 2.1 µl EDTA pH 8.0 [0.5 M], final 20 mM
  - Heat Inactivation (No)
  - Spin Column DNA Purification
  - Gel Electrophoresis and Single Band Excision
  - Phenol-Chloroform Extraction or Ethanol Precipitation.

#### Double Digestion - Buffer Compatibility:

B1 - 25-50 % Relative Activity

B2 - 100 % Relative Activity

B3 - 10-25 % Relative Activity

B4 - 10-25 % Relative Activity

B5 - 50 % Relative Activity

1x UB - 100 % Relative Activity (recommended)

Please note that the optimum digestion condition for this enzyme is 1x UB. Within the Universal Buffer (UB) system, the most majority of our enzymes display 100% Relative Activity in 1x UB and only few either in 0.5x or 2x UB. If optimum condition for second enzyme is different than the recommended for the first enzyme, we suggest carrying out first the restriction at the higher recommended concentration of UB and dilute the reaction volume to the adequate UB concentration for further proceeding with the second restriction.

**FOR RESEARCH USE ONLY**



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#### Reaction Enzymes Buffer Guide:

Buffer 1: 10 × B1  
100 mM Tris-HCl(pH 7.9, 25°C)  
100 mM MgCl<sub>2</sub>  
1000 µg/ml BSA

Buffer 2 10 × B2  
100 mM Tris-HCl(pH 7.9, 25°C)  
100 mM MgCl<sub>2</sub>  
500 mM NaCl  
1000 µg/ml BSA

Buffer 3 10 × B3  
500 mM Tris-HCl(pH 7.9, 25°C)  
100 mM MgCl<sub>2</sub>  
1000 mM NaCl  
1000 µg/ml BSA

Buffer 4 10 × B4  
100 mM Tris-HCl(pH 7.9, 25°C)  
100 mM MgCl<sub>2</sub>  
1500 mM NaCl  
1000 µg/ml BSA

Buffer 5 10 × B5  
200 mM Tris-HCl(pH 7.9, 25°C)  
100 mM Mg acetate  
500 mM K acetate  
1000 µg/ml BSA

#### Reaction Buffer Compatibility:

Our restriction enzymes are fully compatible to restrictases and buffer systems from other manufacturers and can be used along in double digestions. To obtain best results, consult the corresponding manuals of all involved products.

#### DNA Methylation:

No Inhibition: dcm, dam, CpG

#### Quality Control:

All preparations are assayed for contaminating endonuclease, 3'- exonuclease, 5' exonuclease/ 5' phosphatase, as well as nonspecific single- and doublestranded DNase activities.

#### Ligation and recutting:

After 100-fold overdigestion with HindIII, >98% of the DNA fragments can be ligated and recut with this enzyme.

#### Star activity:

Star activity may be observed in the presence of Mn<sup>2+</sup>.