

MBRE011

Pst I

Components

Reagents provided	MBRE011			
	250 Units	500 Units	1000 Units	5000 Units
Pst I	12.5 µl	25 µl	50 µl	250 µl
10X HiBuffer H3	125 µl	250 µl	500 µl	2.5 ml
10X HiBuffer DB	125 µl	250 µl	500 µl	2.5 ml
Diluent E Buffer	62.5 µl	125 µl	250 µl	1.25 ml

NOTE: BSA included in all Reaction Buffer

Source: A *E. coli* strain that carries the Pst I gene from *Providencia stuartii*

Recognition Sequence:



Concentration: 20 U/µl

Unit Definition:

1 u is defined as the amount of enzyme that is required to digest 1µg of DNA in 1 hour at 37°C in 50µl of assay buffer.

Enzyme	Optimum reaction temperature (°C)	Thermal Inactivation (°C)	% activity of Buffers				
			H1	H2	H3	H4	H5
Pst I	37	None	50	75	100	50	50

Reaction Buffer:

10X HiBuffer H3:

50mM Tris -HCl (pH 7.5 at 30°C), 10mM MgCl₂, 100mM NaCl and 100µg/ml BSA.

NOTE: Incubate at 37°C

Storage Buffer:

10mM Tris-HCl (pH 7.5), 300mM NaCl, 0.1mM EDTA, 7mM 2-mercaptoethanol, 200µg/ml BSA and 50% glycerol. Store at -20°C.

NOTE: 10X HiBuffer DB is provided for double digestion.

Quality Control Assays:

Ligation / Recutting Assay:

After 20-fold over digestion with Pst I, more than 95% of the DNA fragments can be ligated and recut.

Over digestion Assay:

An unaltered banding pattern was observed after 1µg of DNA was digested with 40U of Pst I for 16 hours at 37°C.

Example of Digestion conditions:

- Enzyme concentration : 1 Unit
- Lambda DNA 0.3 µg/µl : 3.33 µl (1 µg DNA)
- 10X HiBuffer H3 : 5 µl
- Nuclease free water : upto 50 µl

Note:

- Total reaction volume is dependent on the experiment
- The amount of enzyme to be used is dependent on the DNA template
- For plasmid DNA, 5-10X more enzyme is required
- High enzyme concentration may result in **Star activity**

Storage conditions: Pst I should be stored at -20°C.

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