MBRE004 **Taq I**

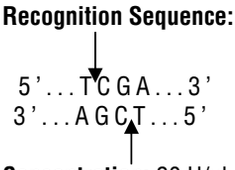
Components

Reagents provided	MBRE004			
	250 Units	500 Units	1000 Units	5000 Units
Taq I	12.5 µl	25 µl	50 µl	250 µl
10X HiBuffer H5	100 µl	200 µl	400 µl	2 ml
10X HiBuffer DB	100 µl	200 µl	400 µl	2 ml
Diluent E Buffer	75µl	150 µl	300 µl	1.5ml

NOTE: BSA included in all Reaction Buffer

Source: A *E.coli* strain that carries the Taq I gene from *Thermus aquaticus*

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
Taq I	65	None	10	100	75	75	100

Reaction Buffer:

10X HiBuffer H5:
30mM Tris-acetate (pH 7.9 at 30°C), 10mM Mg- acetate , 60mM K-acetate, and 100µg/ml BSA .
NOTE: Incubate at 65°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 Taq I, about 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 Taq I for 16 hours at 65°C.

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Example of Digestion conditions:

- Enzyme concentration : 1 Unit
- Lambda DNA 0.3 µg/µl : 3.33 µl (1 µg DNA)
- 10X HiBuffer H5 : 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: Taq I should be stored at -20°C.

MBRE004_/0611 MBRE004-02