

## MBRE008

## Alu I

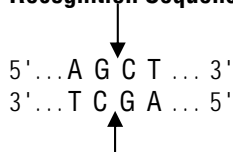
### Components

Reagents provided	MBRE008			
	250 Units	500 Units	1000 Units	5000 Units
Alu I	125 µl	250 µl	500 µl	2.5 ml
10X HiBuffer H5	25 µl	50 µl	0.1 ml	0.5 ml
10X HiBuffer DB	25 µl	50 µl	0.1 ml	0.5 ml
Diluent E Buffer	25 µl	50 µl	0.1 ml	0.5 ml

**NOTE: BSA included in all Reaction Buffer**

**Source:** A *E. coli* strain that carries the Alu I gene from *Arthrobacter luteus*

### Recognition Sequence:



**Concentration:** 2 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 50ml of assay buffer.

Enzyme	Optimum reaction temperature (°C)	Thermal Inactivation (°C)	% activity of Buffers				
			H1	H2	H3	H4	H5
Alu I	37	65	100	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 100 mg/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 2-fold over digestion with Alu I, 70% of the DNA fragments can be ligated and recut.

**Over digestion Assay:**

An unaltered banding pattern was observed after 1 µg of DNA was digested with 4U of Alu I for 16 hours at 37°C.

**Example of Digestion conditions:**

- Enzyme concentration : 1 Unit
- Lambda 0.3 mg/ml : 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:** Alu I should be stored at -20°C.