**MBRE013** **Sca I**

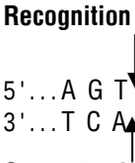
## Components

| Reagents provided | MBRE013 | | | |
|---|---|---|---|---|
| | 250 Units | 500 Units | 1000 Units | 5000 Units |
| Sca I | 25 µl | 50 µl | 100 µl | 500 µl |
| 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 Sca I gene from *Streptomyces albus*

**Recognition Sequence:**




**Concentration:** 10 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 |
| Sca I | 37 | 65 | 75 | 75 | 10 | 10 | 75 |

**Reaction Buffer:**
**Buffer 0.5X DB**
12.5mM Tris-acetate (pH 7.6 at 30°C), 5mM Mg-acetate, 50mM K-acetate, 3.5mM 2-mercaptoethanol and 25µg/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 10-fold over digestion with Sca I, 70% of the DNA fragments can be ligated and recut.

**Over digestion Assay:**
An unaltered banding pattern was observed after 1mg of DNA was digested with 20U of Sca I for 16 hours at 37°C.



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Registered Office :
23, Vadhani Industrial Estate,LBS Marg,
Mumbai - 400 086, India.
Tel. : (022) 4017 9797 / 2500 1607
Fax : (022) 2500 2286

Commercial Office
A-516, Swastik Disha Business Park,
Via Vadhani Indl. Est., LBS Marg,
Mumbai - 400 086, India

Tel: 00-91-22-6147 1919
Fax: 6147 1920, 2500 5764
Email : info@himedialabs.com
Web : www.himedialabs.com

**Example of Digestion conditions:**

- Enzyme concentration : 1 Unit
- Lambda 0.3 mg/ml : 3.33 µl (1 mg DNA)
- 10X HiBuffer DB : 2.5 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:** Sca I should be stored at -20°C.

MBRE013_/0611 MBRE013-02