

## RapidDigest MboII

Cat No.	Digestion site	Quantity	Isoschizomer
RD1181	5'...G A A G A (N) <sub>8</sub> ↓...3' 3'...C T T C T (N) <sub>7</sub> ↑...5'	25μl (1RDU/μl)	-
<b>Source:</b>	<b>Incubation</b>	<b>Inactive</b>	<b>Active site on λ DNA</b>
<i>Moraxella bovis</i>	30 min at 37°C	20 min at 65°C	130

Supplied with: **125μl of 10X RD Universal Buffer, 50μl 10X RD Blue Buffer**

**Store at -20°C**, avoid frequent thawing and freezing.

For in vitro use only

All RapidDigest Restriction Enzymes is completely active in Universal RD Buffer and digest DNA in 15-30 minutes or less.

RD restriction enzyme also eliminates need for sequential digestion during double digest methods.

### Recommended assay

1-Add below materials to 0.5ml tube:

	Plasmid/ Lambda DNA	PCR product	Genomic DNA
<b>Water DNase free</b>	15ul	17ul	30ul
<b>10X RapidDigest Buffer</b>	2ul	2ul	5ul
<b>DNA</b>	2ul (up to 1ug)	10ul (0.2ug)	10ul (5ug)
<b>RapidDigest Enzyme</b>	1ul(1 RDU)	1ul(1 RDU)	5ul(5 RDU)
<b>Total Volume</b>	20ul	30ul	50ul

2- Mix gently and spin down.

3- Incubate at 37°C for 15-30 minutes<sup>1</sup>.

4- Inactive the enzyme by heating for 20 min at 65°C<sup>2</sup>.

1. Time of incubation may need to optimization but it could be achieved between 5 to 30 minutes. For digestion of difficult to cleave-DNA, incubation time may extend to one hour.
2. There are some alternative ways to stop the reaction:
  - Addition of EDTA pH 8.0 <0.5M> final 20mM.
  - Spin column DNA purification.
  - Agarose gel extraction.
  - Phenol- Chloroform extraction.
  - Ethanol precipitation.

**Ligation and recutting:**

After 5-fold over digestion with, >95% of the DNA fragments can be ligated and recut with this enzyme.

**DNA Methylation:**

No Inhibition: CpG, dcm

Inhibition (Impaired by overlapping): dam

**Unit Definition:**

One RapidDigest Unit (1 RDU) equal 5u/μl is the amount of enzyme required to completely digest 1μg of Lambda DNA in 15-30 minute in 1X RapidDigest buffer.

**Quality Control:**

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

For double digestion simply add 1ul of each enzyme and scale up the reaction. For different temperature reaction start of the enzymes that requires lower temperature.

**λ DNA used as substrate for unit definition and quality control tests.**

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