

Rapid Digest RsaI

Cat No.	Digestion site	Quantity	Isoschizomer
RD1101	5'...G T↓A C...3' 3'...C A↑T G...5'	100μl(1RDU/μl)	AfaI, CviQI ^m , RsaNI
source	Incubation time	Inactive	Active site on λ DNA
<i>Rhodopseudomonas sphaeroides</i>	30 minutes at 37°C	20 min at 65°C	113

Supplied with: **0.5 ml 10X RD Universal Buffer, 0.2 ml 10X RD Blue Buffer**

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

For in vitro use only

Rapid Digest Restriction Enzyme is completely active in Universal Rapid Digest Buffer. All RD restriction enzymes are able to 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
Water DNase free	15ul	17ul	30ul
10XRapidDigest Buffer	2ul	2ul	5ul
DNA	2ul (up to 1ug)	10ul (.0.2ug)	10ul (5ug)
Rapid Digest Enzyme	1ul	1ul	5ul
Total Volume	20ul	30ul	50ul

2- Mix gently and spin down.

3- Incubate at 37°C for 30 minutes¹.

4- Inactivate the enzyme by heating for 20 min at 65°C².

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

DNA Methylation:

No Inhibition: dam, dcm

Inhibition (Blocked by some combinations of overlapping): CpG

Unit Definition:

One RapidDigest Unit (1 RDU) 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.