vivantis

RESTRICTION ENDONUCLEASE

Product Datasheet

Sph I

5'...GCATGC...3'
3'...CGTACG...5'

Product No : RV1340
Quantity : 200u

UB BUFFER

37°C

Lot :
Expiry Date :
Concentration : 5u/µl
Supplied with : 1ml of 10X Buffer UB
0.5ml Diluent Viva Buffer A
(BSA included in all Reaction Buffer)
Store at -20°C

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Reaction Conditions:
Buffer 1X UB,
25mM Tris-acetate (pH 7.6 at 30°C), 10mM Mg-acetate,
100mM K-acetate, 7mM 2-Mercaptoethanol and 50µg/ml BSA.
Incubate at 37°C.

Dilution: Viva Buffer A
10mM Tris-HCl (pH 7.4 at 25°C), 50mM KCl, 0.1mM EDTA,
1mM DTT, 200µg/ml BSA and 50% glycerol.

Thermal Inactivation: 65°C for 20 minutes

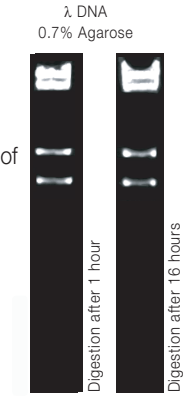
Storage Buffer:
10mM Tris-HCl (pH 7.5), 50mM KCl, 0.1mM EDTA,
7mM 2-mercaptoethanol, 200µg/ml BSA and 50% glycerol.

Unit Definition:
1u 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.

Quality Control Assays:

Ligation/ Recutting Assay:
After 5-fold overdigestion with ***Sph* I**, more than 90% of the DNA fragments can be ligated and recut.

Overdigestion assay:
An unaltered banding pattern was observed after 1µg of DNA was digested with 10u of ***Sph* I** for 16 hours at 37°C.



Activity in Reaction Buffer				
V1	V2	V3	V4	V5
75%	75%	50%	75%	75%

Buffer UB			
0.5X	1.0X	1.5X	2.0X
100%	100%	75%	50%

NOTE:

* Total reaction volume dependent on experiment.
* The amount of enzyme to be used is very much dependent on the DNA template.
* For plasmid DNA, 5-10X more enzyme is required.

Example of Digestion Reaction

Enzyme	:	1 unit
Lambda 0.3µg/µl	:	3.33µl (1µg DNA)
10X Reaction Buffer	:	5µl
Sterile Distilled Water	:	Up to 50µl