vivantis

RESTRICTION ENDONUCLEASE

Product Datasheet

Afi I (*Bsi*Y I)

5'...CCNNNNNNNGG...3'
3'...GGNNNNNNNCC...5'

Product No	:	RV1116
Quantity	:	500u

Lot	:	
Expiry Date	:	
Concentration	:	10u/µl
Supplied with	:	1ml of 10X Buffer V4 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 V4,
10mM Tris-HCl (pH 8.5 at 30°C), 10mM $MgCl_2$,
100mM KCl, and 100µg/ml BSA.
Incubate at 50°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: 80°C for 20 minutes

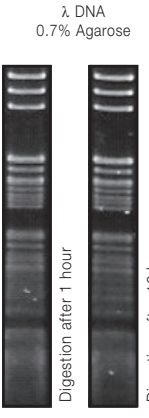
Storage Buffer:
10mM Tris-HCl (pH 7.5), 100mM KCl, 0.15% Triton-X,
0.1mM EDTA, 1mM DTT, 200µg/ml BSA and 50% glycerol.

Unit Definition:
1u is defined as the amount of enzyme that is required to digest 1µg of DNA in 1 hour at 50°C in 50µl of assay buffer.

Quality Control Assays:

Ligation/ Recutting Assay:
After 10-fold overdigestion with ***Afi* I**, 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 20u of ***Afi* I** for 16 hours at 50°C.



Activity in Reaction Buffer				
V1	V2	V3	V4	V5
100%	75%	10%	100%	100%

Buffer UB			
0.5X	1.0X	1.5X	2.0X
10%	10%	50%	25%

* Buffer UB is provided for double digestion purpose.

NOTE:

* Overdigestion in Buffer V4 will cause **Star Activity.**
* 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