


Ava II

 Isoschizomers: *Afl*I, *Bme*18 I, *Eco*47 I, *Sin*I, *Vpa*K11B I

Cat. No.	Amount
EN-E2007-01	1000 Units
EN-E2007-02	5 x 1000 Units

5'... G ↓ G W C C ...3'
 3'... C C W G ↑ G ...5'

Unit Definition: One unit is the amount of enzyme required to completely digest 1 μ g of Lambda DNA in 1 hour in a total reaction volume of 50 μ l. Enzyme activity was determined in the recommended reaction buffer.

For in vitro use only!

Shipping: shipped on blue ice

Storage Conditions: store at -20 °C

Additional Storage Conditions: avoid freeze/thaw cycles

Shelf Life: 12 months

Form: liquid (Supplied in 10 mM Tris-HCl pH 7.5, 50 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 200 μ g/ml BSA and 50 % [v/v] glycerol)

Source: *Anabaena variabilis*, recombinant, *E. coli*

Recommended 50 μ l assay

5 μ l	10x Buffer B5
1 - 2 μ g or 10 μ l	pure DNA or PCR product (0.1 - 2 μ g DNA)
10 units	enzyme
fill up to 50 μ l	PCR grade water

Use 1 unit/ μ g DNA, not exceeding 10 % of reaction volume. Add enzyme as last component. Mix components well before adding enzyme. After enzyme addition, mix gently by pipetting. Do not vortex. High (excess) amounts of enzyme can greatly speed up the reaction. To obtain complete digestion of high molecular weight DNA, (e.g. plant genomic DNA), add excess amounts of enzyme and prolong the incubation time.

Incubate for 1 h at 37 °C.

Stop reaction by alternatively

- Addition of 2.1 μ l EDTA pH 8.0 [0.5 M], final 20 mM
- Heat Inactivation (20 min. at 65 °C)
- Spin Column DNA Purification (e.g. PCR Purification Kit, Cat.-No. PP-201S/L)
- Gel Electrophoresis and Single Band Excision (e.g. Agarose Gel Extraction Kit, Cat.-No. PP 202 S/L)
- Phenol-Chloroform Extraction or Ethanol Precipitation.

Double Digestion - Buffer Compatibility:

B1 - 50 % Relative Activity

B2 - 50 % Relative Activity

B3 - 10 % Relative Activity

B5 - 100 % Relative Activity (recommended)

10x Reaction Buffer B5:

200 mM Tris-acetate (pH 7.9 at 25°C), 100 mM Mg-acetate, 500 mM K-acetate, 10 mM DTT and 1 mg/ml BSA.

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To compensate for the lack of enzyme activity, increase the amount of enzyme and / or reaction time accordingly. The following values may serve as orientation:

- Enzyme amount: Instead of 1 unit of enzyme, use 4 units in buffers providing 25 % relative activity, 2 units in 50 %, 1.5 units in 75 % or 1 unit in 100 %, respectively.
- Reaction time: Increase by 1.3-fold (75 % relative activity), 2 fold (50 %) or 4 fold (25 %), respectively.

Reaction Buffer Compatibility:

Both, enzyme and buffers are fully compatible to restrictases and buffer systems from other manufacturers and can be used along in double digestions. To obtain best results, consult the corresponding manuals of all involved products.

DNA Methylation:No Inhibition: *dam*, *Eco*KIPotential inhibition: *dcm*, CpG**Quality Control:**

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