

AcvI

5'-C A C G T G-3'
3'-G T G C A C-5'

Cat. No.	Size
E2005-01	1 500 units
E2005-02	7 500 units

Reaction Temperature: 37°C

Inactivation Temperature (20 min): 80°C

Prototype: PmaCI

Source: *Aeromonas caviae*

Package Contents:

- **AcvI**
- **10x Reaction Buffer Acet**
- **BSA [100x]**
Added as separate component to prevent reaction buffer precipitation.
- **Dilution Buffer # 1**
Added for enzymes exceeding 10 U/μl in concentration. High protein concentration warrants optimal stability during prolonged storage. Use dilution buffer to prepare short term working stocks (5-10 U/μl, non-freezing at -20°C).

Storage Conditions: Store at -20°C

Double Digestion – Buffer Compatibility:

Buffer	% Rel. Activity
Low	75
Medium	100
High	75
Acet	100

Recommended Buffer: Acet
(or compatible third party buffers)

Restriction Enzyme 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, dcm
Potential inhibition: EcoKI
Inhibition (Blocking): CpG

Standard Reaction Protocol:

Mix the following reaction components:
1-2 μg pure DNA or 10 μl PCR product (≈0.1-2 μg DNA)
5 μl 10x Buffer Acet
0.5 μl BSA [100x]
1-2 U AcvI (use 1 U / μg DNA, < 10 % React. Volume!)
Tips: Add enzyme as last component. Mix components well before adding enzyme. After enzyme addition, mix gently by pipetting. Do not vortex.
@ 50 μl H₂O, nuclease free

Incubate for 1 h at 37°C

Stop reaction by alternatively
(a) Addition of 2.1 μl EDTA pH 8.0 [0.5 M], final 20 mM *or*
(b) Heat Inactivation
20 min at 80°C *or*
(c) Spin Column DNA Purification
(e.g. EURx PCR/DNA CleanUp Kit, Cat.No. E3520) *or*
(d) Gel Electrophoresis and Single Band Excision
(e.g. EURx AgaroseOut DNA Kit, Cat.No. E3540) *or*
(e) Phenol-Chloroform Extraction or Ethanol Precipitation.

Non-optimal buffer conditions:

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:

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

Notes:

It is **not recommended:**

- to perform digestion for over 4 hours;
 - to use more than 10 units of enzyme per 1 μg of DNA.
- These conditions may result in star activity.

Unit Definition:

One unit is the amount of enzyme required to completely digest 1 μg of Ad-2 DNA in 1 hr. Total reaction volume is 50 μl. Enzyme activity was determined in the recommended reaction buffer.

Reaction Buffer:

1 x Acet Buffer: 20 mM Tris-acetate (pH 7.5 at 37°C), 10 mM magnesium acetate, 50 mM potassium acetate, 1 mM dithiothreitol.

To be supplemented with 100 μg/ml bovine serum albumin.

Storage Buffer:

10 mM Tris-HCl (pH 7.5 at 22°C), 0.1 mM dithiothreitol, 50 mM KCl, 10 mM MgCl₂, 200 μg/ml bovine serum albumin and 50 v/v) glycerol.

Quality Control:

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