

## SnapDigest Alw44I

FB02220020 200 µL (200 Rxns)

Store at -25°C to -15°C

5' G ↓ T G C A C 3'  
3' C A C G T ↑ G 5'

SnapDigest Alw44I restriction enzyme recognizes G<sup>^</sup>TGCAC site and cuts best at 37°C in 5-15 minutes using universal SnapDigest Buffer.

SnapDigest enzymes are advanced restriction enzymes allowing rapid DNA digestion in just 5-15 minutes. They exhibit 100% activity in the universal SnapDigest and SnapDigest Green buffers, enabling simultaneous digestion with multiple enzymes in one tube without needing sequential steps. These enzymes work on plasmid, genomic, viral DNA, and PCR products without star activity, even during long incubations. Common downstream enzymes (for ligation, etc.) are also fully active in these buffers.

SnapDigest Green Buffer contains tracking dyes (blue ~3-5 kb, yellow <10 bp in 1% agarose) and a density reagent for direct gel loading. However, these dyes may interfere with fluorescence measurements, so the standard SnapDigest buffer is recommended for applications requiring fluorescence analysis.

### Kit Contents

Reagents	Volume
SnapDigest Alw44I	200 µL
10X SnapDigest Buffer	1.0 mL
10X SnapDigest Green Buffer	1.0 mL

## SnapDigest Protocol of Different DNA

1. Combine the following reaction components at room temperature in order:

Component	Plasmid DNA	PCR Product	Genomic DNA
Water, nuclease-free	15 µL	17 µL	30 µL
10X SnapDigest or 10X SnapDigest Green Buffer	2 µL	2 µL	5 µL
DNA	2 µL (up to 1 µg)	10 µL (~0.2 µg)	10 µL (5 µg)
SnapDigest Enzyme	1 µL	1 µL	5 µL

- Mix gently and spin down.
- Incubate at 37°C in a heat block or water thermostat for 5 minutes (genomic DNA), or for 10 minutes (PCR product), or for 60 minutes (plasmid DNA). (Optional: Inactivate the enzyme by heating for 5 minutes at 80°C.)
- If SnapDigest Green Buffer was used in the reaction, load an aliquot directly on a gel.

### Double or Multiple Digestion of DNA

- The combined volume of enzymes should not exceed 1/10 of the total reaction volume.
- Use 1 µL of each enzyme and scale up the reaction conditions appropriately.
- If the enzyme requires different ion reaction temperatures, start with the enzyme with the lower temperature requirement then add the second enzyme and incubate at the higher temperature.

### Scaling up Plasmid DNA Digestion Reaction

DNA	1 µg	2 µg	3 µg	4 µg	5 µg
SnapDigest Enzyme	1 µL	2 µL	3 µL	4 µL	5 µL
10X SnapDigest or 10X SnapDigest Green Buffer	2 µL	2 µL	3 µL	4 µL	5 µL
Total Volume	20 µL	20 µL	30 µL	40 µL	50 µL

Increase the incubation time by 3-5 minutes if the total volume exceeds by 20  $\mu$ L.  
Use water Thermostat, air thermostat are not recommended due to the slow transfer of heat to the reaction mixture.

### Recommendations for PCR Product Digestion

Purify PCR products before restriction digestion in the following scenarios:

- When PCR additives (e.g., DMSO or glycerol) were used:  
Purification removes components that may inhibit cleavage efficiency or induce star activity.
- When PCR products are intended for cloning:  
Purification eliminates residual active thermostable DNA polymerase, preventing it from adversely modifying digested DNA ends and ensuring optimal ligation efficiency.