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Avr II

1 Contents

Component	HY-KE7002-25T			
Avr II	25 µL			
10× Buffer	1 mL			
10× Color Buffer	1 mL			

2 Introduction

Avr II is a restriction enzyme for rapid DNA digestion, including plasmid, genomic DNA as well as PCR products. Isoschizomers: AspA2 I, Bln I, XmaJ I. Avr II is 100% active in 10× Buffer and 10× Color Buffer. 10× Color Buffer includes a density reagent along with red and yellow tracking dyes that allow for direct loading of the reaction mixtures on a gel. The red dye of the Color Buffer migrates with 2500 bp DNA fragments in a 1% agarose gel and the yellow dye of the Color Buffer migrates faster than 10 bp DNA fragments in a 1% agarose gel.

Cleavage site

5'....C T A G G....3'

3'...G G A T C C...5'

3 General Protocol

1. Fast Digestion of Different DNA

1.1 Combine the following reaction components on ice in the order indicated:

	Plasmid DNA	PCR product	Genomic DNA
ddH ₂ O	15 µL	16 µL	30 µL
10× Buffer or 10× Color Buffer	2 µL	3 µL	5 µL
DNA	2 µL (up to 1 µg)	10 μL (~ 0.2 μg)	10 μL (5 μg)
Avr II	1 µL	1 µL	5 µL
Total	20 µL	30 µL	50 µL

Note: When PCR product will be used for cloning, it is recommended to purify PCR product prior digestion.

1.2 Mix gently and spin down.

1.3 Incubate at 37°C for 15 min (Plasmid DNA), or for 15-30 min (PCR product), or for 30-60 min (Genomic DNA).

1.4 (Optional) Inactivation: Phenol/chloroform extraction and ethanol precipitation of DNA. Thermal inactivation is not applicable.

1.5 If 10× Color Buffer was used in the reaction, load an aliquot of the reaction mixture directly on a gel.

2. Scaling up Plasmid DNA Digestion Reaction

DNA	1 µg	2 µg	3 µg	4 µg	5 µg
Avr II	1 μL	2 µL	3 µL	4 μL	5 μL
10× Buffer or 10× Color Buffer	2 µL	2 µL	3 µL	4 µL	5 μL
Total	20 µL	20 µL	30 µL	40 µL	50 μL

Note: Increase the incubation time by 3-5 min if the total reaction volume exceeds 20 µL. It is recommended to use heat block or water thermostat.

4 Number of Recognition Sites in DNA

λDNA	ФХ174	pBR322	pUC57	pUC18/19	SV40	M13mp18/19	Adeno2
2	0	0	0	0	2	0	2

5 Methylation Effects on Digestion

Dam	am Dcm		EcoKI	EcoBI
no effect				

6 Storage

-20°C, 2 years.

7 Precautions

1. The combined volume of the enzymes in the reaction mixture should not exceed 1/10 of the total reaction volume.

2. Use 1 µL of each enzyme and scale up the reaction conditions appropriately.

3. If the enzymes require different reaction temperatures, start with the enzyme that requires a lower temperature, then add the second enzyme and incubate at the higher temperature.

4. This product is for R&D use only, not for drug, household, or other uses.

5. For your safety and health, please wear a lab coat and disposable gloves to operate.