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Cla I

1 Contents

Component	HY-KE7010-50T
Cla I	50 μL
10× Buffer	1 mL
10× Color Buffer	1 mL

2 Introduction

Cla I is a restriction enzyme for rapid DNA digestion, including plasmid, genomic DNA as well as PCR products. Isoschizomers: BspD I, Bsa29 I, BseC I, Bsu15 I, BsuTU I. Cla I 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'...A T▼C G A T...3'

3'...T A G C▲T A...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_2O	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)
Cla I	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) Inactivate the enzyme by heating for 20 min at 80°C.

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
Cla I	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	ΦX174	pBR322	pUC57	pUC18/19	SV40	M13mp18/19	Adeno2
15	0	1	0	0	0	2	2

5 Methylation Effects on Digestion

Dam	Dcm	CpG	EcoKI	EcoBI
blocked	no effect	blocked	no effect	blocked

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.