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EcoR I, ADCF

1 Contents

Component	HY-KE7053-5000U
EcoR I, ADCF (20 U/μL)	5000U
10× EcoR I Buffer (ADCF)	1 mL × 2

2 Introduction

EcoR I, ADCF is a restriction enzyme for rapid DNA digestion, including plasmid, genomic DNA as well as PCR products. This product is animal derived component free, and has good redundancy of enzyme activity, which can easily cope with excessive substrate or difficult template enzyme cutting. Isoschizomers: Fun II.

Cleavage site

5'...G▼A A T T C...3'

3'...C T T A A ▲G...5'

3 General Protocol

1. Digestion of DNA

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

ddH_2O	to 50 μL
10× EcoR I Buffer (ADCF)	5 μL
DNA	1 μg
EcoR I, ADCF	10-20 U
Total	50 μL

Note: DNA should be free of contaminants such as phenol, chloroform, alcohol, EDTA, detergents or excessive salts.

1.2 Mix gently and spin down.

1.3 In general, we recommend 5–10 U of enzyme per μg plasmid DNA or PCR product, and 10–20 U for genomic DNA in a 15-60 min digest at 37°C.

1.4 (Optional) Inactivate the enzyme by heating for 20 min at 80°C. Or remove enzyme by using a spin column or phenol/chloroform extraction.

2. Scaling down DNA Digestion Reaction

DNA	0.1 μg	0.5 μg
EcoR I, ADCF	1 U	5 U
10× EcoR I Buffer (ADCF)	1 μL	2.5 μL
ddH_2O	to 10 μL	to 25 μL
Total	10 μL	25 μL

Note: 10 μL reaction mix should not be incubated for longer than 1 hour to avoid evaporation.

4 Unit Definition

One unit is defined as the amount of EcoR I, ADCF required to digest 1 μg of λDNA in 1 hour at 37°C in a total reaction volume of 50 μL.

5 Number of Recognition Sites in DNA

λDNA	ΦX174	pBR322	pUC57	pUC18/19	SV40	M13mp18/19	Adeno2
5	0	1	1	1	1	1	5

6 Methylation Effects on Digestion

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

7 Storage

-20°C, 2 years.

8 Precautions

1. Enzyme volume should not exceed 10% of the total reaction volume to prevent star activity due to excess glycerol.
2. Additives in the restriction enzyme storage buffer (e.g., glycerol, salt) as well as contaminants found in the substrate solution (e.g., salt, EDTA, or alcohol) can be problematic in smaller reaction volumes.
3. This product is for R&D use only, not for drug, household, or other uses.
4. For your safety and health, please wear a lab coat and disposable gloves to operate.