



Restriction Enzyme EcoR V



Cat.#	Size	Conc.
FG-EcoRV	4,000 units	20 units/μl

Store at -20°C

Supplied with: 10X FastGene® Buffer III (FG-REB3)
10X FastGene® FastCut Buffer (FG-REBHF)
6X DNA Loading Buffer
Sterile water

Recognition site



For Research Use Only. Not for use in diagnostic procedures.



Source: *E. coli* that carries the EcoR V gene from plasmid J62 pLG74

Reaction conditions

1X FastGene® Buffer III, 37°C
1X FastGene® FastCut Buffer, 37°C

FastGene® FastCut Buffer

FastGene® restriction enzyme can cut substrate DNA in 5-15 min with FastGene® FastCut Buffer.

1X FastGene® Buffer III

50 mM Tris-HCl (pH 7.9 at 25°C)
100 mM NaCl
10 mM MgCl₂
100 μg/ml BSA

Unit definition

One unit is defined as the amount of enzyme required for complete digestion of 1 μg bacteriophage λ at 37°C for 1 hr in 50 μl reaction mixtures.

Quality control

- Unit definition assay
- Overdigestion assay
- Endonuclease assay
- Extreme pure assay

Dilution buffer

FastGene® Diluent A

Heat Inactivation

EcoR V can be inactivated at 65°C for 20 min.

Methylation sensitivity

dam methylation: Not sensitive
dcm methylation: Not sensitive
CpG methylation: Conditionally sensitive

Prolonged incubation

A minimum amount of enzyme required to digest 1 μg substrate DNA for 16 hr; 0.5 U.

Relative activity in FastGene® Buffers

FastGene® Buffer I:	0%
FastGene® Buffer II:	100%
FastGene® Buffer III:	100%
FastGene® Buffer IV:	50%
FastGene® FastCut Buffer:	100%

Note

No further activity is noted after 4 hr incubation at 37°C and thus overnight incubation is not effective. Cleavage of mammalian DNA is blocked by overlapping CpG methylation. A single EcoR V site is found in the tetracycline resistance gene of pBR322.

Standard reaction condition

- Normal protocol

Component	Final Conc.	Volume
Substrate DNA	1 μg	X μl
10X FastGene® Buffer III	1 X	5 μl
EcoR V	20 unit	1 μl
Sterile water		up to 50 μl

→ Incubate at 37°C for 1 hr

- Fast protocol

Component	Final Conc.	Volume
Substrate DNA	1 μg	X μl
10X FastGene® FastCut Buffer	1 X	5 μl
EcoR V	20 unit	1 μl
Sterile water		up to 50 μl

→ Incubate at 37°C for 15 min

※ We recommend 5-10 units of enzyme per μg DNA and 10-20 units for genomic DNA in a 1 h digest.



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Methylation sensitivity

dam methylation: Not sensitive
dcm methylation: Not sensitive
CpG methylation: Conditionally sensitive

Prolonged incubation

A minimum amount of enzyme required to digest 1 μg substrate DNA for 16 hr; 0.5 U.

Relative activity in FastGene® Buffers

FastGene® Buffer I:	0%
FastGene® Buffer II:	100%
FastGene® Buffer III:	100%
FastGene® Buffer IV:	50%
FastGene® FastCut Buffer:	100%

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EcoR V	20 unit	1 μl
Sterile water		up to 50 μl

→ Incubate at 37°C for 1 hr

- Fast protocol

Component	Final Conc.	Volume
Substrate DNA	1 μg	X μl
10X FastGene® FastCut Buffer	1 X	5 μl
EcoR V	20 unit	1 μl
Sterile water		up to 50 μl

→ Incubate at 37°C for 15 min

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