Version: M17L05V1.1

Eco31I（Bsa I）

Cat. No. : RK21129

Product Components

Components	Component Number	Concentration	1,000 U
Eco31I	RM21630	10,000 U/mL	100 μL
10X Buffer CutS	RM20103	10X	200 μL

Product Description

Restriction Site

5'...G G T C T C(N)$_1$▼...3'

3'...C C A G A G(N)$_5$▲...5'

Unit Definition

One unit is defined as the amount of enzyme required to digest 1 μg of DNA in 1 hour at 37°C in a total reaction volume of 20 μL.

Storage

-20°C

Reaction Conditions

1X Buffer CutS, incubate at 37°C.

Heat Inactivation

80°C for 20 min.

Instructions

Recommended Protocol for Digestion

Components	Volume
ddH_2O	Up to 20 μL
10X Buffer CutS	2 μL
PCR fragment or donor vector	0.2-1 μg
Eco31I	0.5-1 μL

- Mix components by pipetting the reaction mixture up and down, or by "flicking" the reaction tube. Follow with a quick ("touch") spin-down in a microcentrifuge. Do not vortex the reaction.
- Incubate at 37°C for 1 hr.
- Inactivated at 80°C for 20 min. (Optional)

Note

1. Enzyme

- Keep on ice when not in the freezer.
- Should be the last component added to reaction.
- In general, we recommend 5 - 10 units of enzyme per μg DNA, and 10 - 20 units for genomic DNA in a 1 hour digest.

2. DNA

- Should be free of contaminants such as phenol, chloroform, alcohol, EDTA, detergents or excessive salts. Extra wash steps during purification are recommended.
- Methylation of DNA can inhibit digestion with certain enzymes.
- Methylation Sensitivity

Dam	not sensitive
Dcm	may overlap, cleavage impaired
CpG	may overlap, cleavage impaired
EcoKI	not sensitive
EcoBI	may overlap, effect not determined

- Number of Recognition Sites in DNA

pUC57	pUC18/19	pBR322	λDNA
1	1	1	2

3. Buffer

- Use at a 1X concentration.
- Activity in different Buffer

ABclonal CutS	NEB CutSmart Buffer	Thermo Scientific FastDigest Buffer
100%	100%	100%

4. Reaction Volume

- A 20 µL reaction volume is recommended for digestion of 1 µg of substrate.
- Enzyme volume should not exceed 10% of the total reaction volume to prevent star activity due to excess glycerol.
- 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. The following guidelines can be used for techniques that require smaller reaction volumes.

Reaction System	Enzyme Amount*	DNA	10X ABclonal Buffer
10 µL**	1 U	0.1 µg	1 µL
25 µL	5 U	0.5 µg	2.5 µL
50 µL	10 U	1 µg	5 µL

* Note: Restriction Enzymes should be diluted when smaller amounts are needed. ** Note: 10 µL rxns should not be incubated for longer than 1 hour to avoid evaporation.

5. Incubation Time

- Incubation time is typically 1 hour.
- Can often be decreased by using an excess of enzyme, or by using one of our "Quick Cut" restriction enzymes.

6. Stopping a Reaction

If no further manipulation of DNA is required:

- Terminate with a stop solution (10 µL per 50 µL rxn) [1x: 2.5% Ficoll-400, 10 mM EDTA, 3.3 mM Tris-HCl, 0.08% SDS, 0.02% Tartrazine, 0.001% Xylene Cyanol FF, pH 8.0 @ 25°C].

When further manipulation of DNA is required:

- Heat inactivation can be used.
- Remove enzyme by using a spin column or phenol/chloroform extraction.

7. Recommended Protocol for One-step ligation reactions

Components	Amount
Nuclease-free water	to 20 µL
Inserts*	0.1 pmol
Plasmid	0.05 pmol
10x T4 DNA ligase Reaction buffer	2 µL
T4 DNA Ligase (High Conc.) (RK21500)	1 µL
Eco31I	1 µL

* Note: When multiple inserts are present, 0.1 pmol is invested for each inserts.

- **Assembly Protocol for 1 Insert**

Temperature	Time	Cycle
37°C	10 min	1
60°C	5 min	1

- **Assembly Protocol for 2+ Inserts**

Temperature	Time	Cycle
37°C	1 min	30
16°C	1 min	
60°C	5 min	1

8. Control Reactions

If you are having difficulty cleaving your DNA substrate, we recommend the following control reactions:

- Control DNA (DNA with multiple known sites for the enzyme, e.g. lambda or pUC19 DNA) with restriction enzyme to test enzyme viability.
- If the control DNA is cleaved and the experimental DNA resists cleavage, the two DNAs can be mixed to determine if an inhibitor is present in the experimental sample. If an inhibitor (often salt, EDTA or phenol) is present, the control DNA will not cut after mixing.