

Product Components

| Components | Component Number | 4,000 U |
|---------------------|------------------|---------|
| PvuII (20,000 U/mL) | RM21668 | 200 µL |
| 10X Buffer CutS | RM20103 | 1.25 mL |

Product Description

Restriction Site



Unit Definition

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

Storage

-20°C

Reaction Conditions

1X Buffer CutS, incubate at 37°C.

1X Buffer CutS

50 mM KAc, 20 mM Tris-HAc, 10 mM MgAc₂,
 100 µg/mL rHSA, pH 7.9 @ 25°C

Quick Cut

Yes. This enzyme will digest unit substrate in 5-15 minutes under recommended reaction conditions.

Heat Inactivation

No

Methylation Sensitivity

| dam methylation | dcm methylation | CpG Methylation |
|-----------------|-----------------|-----------------|
| not sensitive | not sensitive | not sensitive |

Instructions

Recommended Protocol for Digestion

| Components | 50 µL |
|--------------------|-------------|
| ddH ₂ O | Up to 50 µL |
| 10X Buffer CutS | 5 µL |
| DNA* | 1 µg |
| PvuII | 1 µL |

* Note: DNA substrates should be free of phenol, chloroform, ethanol, EDTA, detergents or high concentrations of salt, otherwise it will affect the enzyme activity.

- ◆ The substrates are completely digested in 5-15 min incubate at 37°C.
- ◆ For enzymes that cannot be heat-inactivated, we recommend using a column for cleanup, or running the reaction on an agarose gel and then extracting the DNA, or performing a phenol/chloroform extraction.

QC Process

- ◆ Purity is above 95% detected by SDS-PAGE.
- ◆ No exonuclease, nuclease contamination.
- ◆ No residual host genomic DNA detected by PCR.

Optimizing Restriction Endonuclease Reactions

There are several key factors to consider when setting up a restriction endonuclease digest. Using the proper amounts of DNA, enzyme and buffer components in the correct reaction volume will allow you to achieve optimal digestion. Most researchers follow the "typical"

reaction conditions listed, where a 5 - 10 fold overdigestion is recommended to overcome variability in DNA source, quantity and purity.

A "Typical" Restriction Digest

| | |
|------------------------|---|
| Restriction Enzyme | 10 units is sufficient, generally 1 μ L is used |
| DNA | 1 μ g |
| 10X ABclonal Buffer | 5 μ L (1X) |
| Total Reaction Volume | 50 μ L |
| Incubation Time | 1 hr* |
| Incubation Temperature | Enzyme dependent |

* Note: Can be decreased to 5-15 minutes by using a "Quick Cut" Restriction Enzyme.

1. Enzyme

- In general, we recommend 5 - 10 units of enzyme per μ g DNA, and 10-20 units for genomic DNA in a 1 hour digest.

2. DNA

- Methylation of DNA can inhibit digestion with certain enzymes.

3. Buffer

- Use at a 1X concentration.
- Supplement with SAM (S-Adenosyl methionine) to the recommended concentration if required.

4. Reaction Volume

- A 50 μ L reaction volume is recommended for digestion of 1 μ g 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 μ L** | 1 U | 0.1 μ g | 1 μ L |
| 25 μ L | 5 U | 0.5 μ g | 2.5 μ L |
| 50 μ L | 10 U | 1 μ g | 5 μ L |

* Note: Restriction Enzymes should be diluted when smaller amounts are needed.

** Note: 10 μ L 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.
- It is possible, with many enzymes, to use fewer units and digest for up to 16 hours.

6. Stopping a Reaction

If no further manipulation of DNA is required:

- Terminate with a stop solution (10 μ L per 50 μ L 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. 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.