

## Product components

Components	Component number	Concentration	Size-1	Size-2
			1,000 U	5,000 U
T7 Exonuclease	RM20566	10,000 U/mL	100 µL	500 µL
10X ABuffer S	RM20147	10X	1.25 mL	2 × 1.25 mL

## Product Description

T7 Exonuclease is a double-stranded DNA-specific exonuclease that degrades linear or nicked double-stranded DNA in the 5' to 3' direction. The enzyme does not digest supercoiled dsDNA.

## Product Source

The T7 Exonuclease gene is expressed and purified from *E. coli*.

## Storage

-20°C

## Unit Definition

One unit is defined as the amount of enzyme required to produce 1 nmol of acid-soluble deoxyribonucleotide from 0.15 mM sonicated [<sup>3</sup>H]-DNA in a total reaction volume of 50 µL in 30 minutes at 37°C in 1X ABuffer S.

## Reaction Conditions

1X ABuffer S, Incubate at 25°C

## 1X ABuffer S

20 mM Tris-acetate, 50 mM Potassium Acetate, 10 mM Magnesium Acetate, 1 mM DTT, pH 7.9 @ 25°C

## Storage Conditions

10 mM Tris-HCl, 0.1 mM EDTA, 5 mM DTT, 50% glycerol, pH 8 @ 25°C

## Heat Inactivation

No

## Operation Description

1. Set-up the following reaction on ice, noting that the enzyme should be added at the end and then gently mix the reaction by pipetting up and down and microcentrifugation.

Components	50 $\mu$ L Reaction
10X ABuffer S	5 $\mu$ L(1X)
DNA	Variable( up to 1 $\mu$ g)
T7 Exonuclease	1 $\mu$ L(10 U)
Nuclease-Free-Water	up to 50 $\mu$ L

2. Incubate at 25°C for 30 minutes.

3. Terminate reaction by adding EDTA to at least 11 mM.

4. To purify digested samples, we recommend using one of the following steps:

① Column clean up or Agarose gel recovery.

② Performing a phenol/chloroform extraction followed by ethanol precipitation.

*Note: For more precise results or partial digestion reactions, it is recommended to determine the specific enzyme-to-substrate ratio through titration of the enzyme and substrate.*