

T7 Gene 6 Exonuclease

Product number 70025

Digestion protocol

T7 Gene 6 Exonuclease hydrolyzes duplex DNA non-processively in the 5'→3' direction from both 5'-phosphoryl or 5'-hydroxyl nucleotides by liberating mononucleotides until about 50% of the DNA is acid soluble. It also degrades nucleotides at the gaps and nicks of double-stranded DNA from the 5' termini and RNA from RNA:DNA hybrids from the 5'→3' direction.

Properties:

Molecular weight: 32,000 Da

Optimum pH: 7.5

Optimum temperature: 37°C

T7 Gene 6 Exonuclease and 5X T7 Gene 6 Exonuclease Reaction Buffer are functionally tested in the following protocol to convert 0.5 pmol of λ DNA to single-stranded half molecules using 75 units of enzyme in 30 minutes at 37°C.

5X T7 Gene 6 Exonuclease Reaction Buffer: 200 mM Tris-HCl (pH 7.5), 100 mM MgCl₂, 250 mM NaCl.

Digestion of double-stranded DNA with T7 Gene 6 Exonuclease

DNA (~2.5 µg/µl) _____ µl

5X T7 Gene 6 Exonuclease Buffer 3 µl

T7 Gene 6 Exonuclease (5 units/µg DNA) _____ µl

Water to 15 µl

Incubate at 37°C for 30 minutes.

Incubate at 80°C for 10 minutes to inactivate the enzyme.

Products should be checked on an agarose gel. To avoid trapping of DNA in the wells of agarose gels or "sticky-end" artifact bands, heat samples to 65–75°C for 3–4 minutes prior to loading.

Note: The above buffer is a "medium salt" buffer, which works for many restriction enzymes but may not work for all of them. If a specific buffer is used for digestion, subsequent digestion with T7 Gene 6 Exonuclease usually still works satisfactorily. Subsequent reactions should be carried out in the appropriate buffer. Ethanol precipitation after digestion may be used to change buffers.

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Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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