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 $MgCl_2$, 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.

Documentation and support

Customer and technical support

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  - User guides, manuals, and protocols
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  - Safety Data Sheets (SDSs; also known as MSDSs)

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

Limited product warranty

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