Incubation of a 10 µl reaction
1X RNase H Reaction Buffer.
Incubation of a 50 µl
1X RNase H Reaction Buffer.
Incubation of a 50 µl
Ribonuclease H (RNase) H is an
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One unit is defined as the amount
Ribonuclease H (RNase) H is an
Source: An E. coli strain that carries the cloned
RNase H gene (rnh) from Escherichia coli
Applications:
• Removal of poly(A) tails of mRNA hybridized to poly(dT)
• Removal of mRNA during second strand cDNA synthesis
Supplied in: 50 mM KCl, 10 mM Tris-HCl (pH 7.4 @ 25°C), 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml BSA and 50% glycerol.
Reagents Supplied with Enzyme:
10X RNase H Reaction Buffer.
Reaction Conditions: 1X RNase H Reaction Buffer. Incubate at 37°C.
1X RNase H Reaction Buffer:
75 mM KCl
50 mM Tris-HCl
3 mM MgCl
10 mM dithiothreitol
pH 8.3 @ 25°C
Unit Definition: One unit is defined as the amount of enzyme required to produce 1 nmol of ribonucleotides from 20 picomoles of a fluorescently labelled 50 base pair RNA-DNA hybrid in a total reaction volume of 50 µl in 20 minutes at 37°C.
Quality Control Assays
RNase Assay: Incubation of a 10 µl reaction containing 50 units of RNase H with 40 ng of RNA transcripts for 1 hour at 37°C results in no detectable degradation of the RNA as determined by gel electrophoresis.
SS DNA Exonuclease Activity: Incubation of 50 units of enzyme with 1 µg of sonicated and denatured [3H]-DNA (10^4 cpmp/µg) for 30 minutes at 37°C in 50 µl reaction buffer released < 0.1% radioactivity.
Endonuclease Activity: Incubation of a 50 µl reaction containing 50 units of RNase H with 1 µg of qX174 RF I DNA for 4 hours at 37°C resulted in < 10% conversion to RFII as determined by agarose gel electrophoresis.
Heat Inactivation: 65°C for 20 minutes.
References:

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