

User Manual

Version 3.0

Product name: RNase H, *E. coli*

Cat #: RNHE-100, RNHE-200, RNHE-OEM, B-RH10

Description:

RNase H(*rnh*, *E. coli*) is an endoribonuclease which degrades the RNA strand of RNA/DNA hybrid molecules. RNase H digestion produces ribonucleotide molecules with 5'-phosphate and 3'-hydroxyl termini. RNase H is nearly inactive against single or double-stranded RNA molecules.

Protocol:

Protocol for Second Strand cDNA Synthesis

Perform first strand cDNA synthesis reaction according to recommendations provided for a specific reverse transcriptase.

Set up the following (on ice) first strand cDNA synthesis reaction mixture:

10X reaction buffer for DNA Polymerase I 4 μ l

RNase H 0.2 μ l (1U)

DNA Polymerase I 1.5 μ l (30U)

To total volume 50 μ l

Gently vortex and briefly centrifuge.

Incubate at 15°C for 2 hours. Do not let the temperature rise above 15°C.

Add 2.5 μ l (12.5U) of T4 DNA Polymerase and incubate at 15°C for 5 min.

Terminate the reaction by adding 5 μ l of 0.5 M EDTA, pH 8.0.

cDNA can be used for further cloning related procedures.