RNase

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Ribonuclease A (RNase A)

A pancreatic ribonuclease.
At low salt concentrations (0 to 100mM NaCl), RNase A cleaves single-stranded and double-stranded RNA as well the RNA strand in RNA-DNA hybrids. However, at NaCl concentrations of 0.3M or higher, RNase A specifically cleaves single-stranded RNA

RNAse A specifically cleaves at the 3'side of a pyrimidine (uracil or cytosine) phosphate bonds. Cleavage takes place in two steps: first, the 3',5'-phosphodiester bond is cleaved to generate a 2',3'-cyclic phosphodiester intermediate; second, the cyclic phosphodiester is hydrolyzed to a 3'-monophosphate.
It can be inhibited by ribonuclease inhibitor protein, by heavy metal ions, and by uridine-vanadate complexes.

Ribonuclease H (RNase H) is a non-specific endonuclease and catalyzes the cleavage of RNA via a hydrolytic mechanism.

RNase H's ribonuclease activity cleaves the 3'-O-P bond of RNA in a DNA/RNA duplex to produce 3'-hydroxyl and 5'-phosphate terminated products. In DNA replication, RNase H is responsible for removing the RNA primer, allowing completion of the newly synthesized DNA.

In the laboratory, as RNase H specifically degrades the RNA in RNA:DNA hybrids and will not degrade DNA or unhybridized RNA, it is commonly used to destroy the RNA template after first-strand complementary DNA (cDNA) synthesis by reverse transcription, as well as procedures such as nuclease protection assays.
RNase H can also be used to degrade specific RNA strands when the cDNA oligo is hybridized, such as the removal of the poly(A) tail from mRNA hybridized to oligo(dT), or the destruction of a chosen non-coding RNA inside or outside the living cell.
To terminate the reaction, a chelator, such as EDTA, is often added to sequester the required metal ions in the reaction mixture.

Application
Ribonucelases do not hydrolyse DNA because the DNA lacks the 2'-oh groups necessary for cyclic intermediates.

Provided as a powder or solution; if in powder form make up to 10mg/ml and freeze in aliquots.

Use at 1-100ng/ml at pH 7.0 - 8.5
Include EDTA present at 1-5mM to prevent sample endogenous DNAse activity.

Unit definition is ambiguous so always optimise the amount needed if not advised in the protocol.