RNase HII is cleaved from the fusion construct by Factor Xa and then purified away from both MBP and Factor Xa. RNase HII cleaved from MBP has four additional amino acids at its N-terminus (Ile-Ser-Glu-Phe).

Applications:
- Nicking of products generated with a polymerase that will incorporate ribonucleotides
- Generation of a double-stranded break at the site of an incorporated ribonucleotide when used with T7 Endo I
- Degradation of the RNA portion of Okazaki fragments

Note: RNase HII displays reduced hybridase activity compared to RNase H (2). Okazaki fragments are preferentially nicked 5' to the ribonucleotide at the junction of the RNA/DNA sequence (1). RNase HII prefers a dsDNA duplex containing a single ribonucleotide over a RNA/DNA hybrid substrate (1) or an Okazaki fragment (3).

Supplied in: 100 mM NaCl, 20 mM Tris-HCl (pH 8.0), 1 mM EDTA, 1 mM dithiothreitol and 50% glycerol.

Reagents Supplied with Enzyme:
- 1X ThermoPol Reaction Buffer.

Reaction Conditions: 1X ThermoPol Reaction Buffer. Incubate at 37°C.

1X ThermoPol Reaction Buffer:
- 10 mM KCl
- 20 mM Tris-HCl
- 10 mM (NH₄)_2SO₄
- 2 mM MgSO₄
- 0.1% Triton X-100
- pH 8.8 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme required to yield a fluorescence signal consistent with the nicking of 100 pmol of synthetic double-stranded DNA substrate containing a single ribonucleotide near the quencher of a fluorophore/acceptor pair in 30 minutes at 37°C in 1X ThermoPol Reaction Buffer.

Unit Assay Conditions: 1X ThermoPol Reaction Buffer with 30 nM of a synthetic dsDNA 26-mer containing an internal ribonucleotide in a total reaction volume of 150 µl.

Heat Inactivation: No

Note: Incubation with 0.1% SDS is sufficient to inactivate RNase HII.

Quality Control Assays

RNase Assay: Incubation of a 10 µl reaction containing 50 units of RNase HII with 3.3 pmol of a synthetic RNA oligo (26-mer) for 1 hour at 37°C resulted in no detectable degradation of the RNA as determined by PAGE analysis.

Exonuclease Assay: Incubation of a 50 µl reaction containing 50 units of RNase HII with 1 µg of pBR322 DNA for 4 hours at 37°C resulted in <10% conversion from supercoiled to nicked molecules as determined by agarose gel electrophoresis.

(see other side)
Reaction Protocol:
The following is a typical reaction protocol for nicking at the site of a single ribonucleotide within a dsDNA substrate or for nicking an Okazaki fragment 5’ to the ribonucleotide adjacent to the DNA.

1. Add 100 picomoles of double stranded substrate to 1X ThermoPol Buffer.
2. Bring the reaction volume to 20 µl with water.
3. Add 1 µl of RNaseHII and mix thoroughly.
4. Incubate at 37°C for 15 minutes.

Note: To achieve the best results for a given substrate, it is often helpful to test more than one concentration of ribonuclease.

References: