**RNase If**

*Source:* An *E. coli* strain containing a genetic fusion of the RNase I gene (rna) from *E. coli* and maltose-binding protein. It has identical activity to RNase I.

**Description:** Ribonuclease If (RNase If) is a single strand specific RNA endonuclease which will cleave at all RNA dinucleotide bonds leaving a 5’ hydroxyl and 2’, 3’ cyclic monophosphate (1). RNase If is a recombinant protein fusion of RNase I (from *E. coli*) and maltose-binding protein. It has identical activity to RNase I.

**Unit Definition:** One unit is defined as the amount of enzyme required to fully digest 1 picomole of synthetic ssRNA 33-mer in a total reaction volume of 10 µl in 15 minutes in 1X NEBuffer 3 as visualized on a 20% acrylamide gel (40:1 Bis) stained with SYBR Gold®.

**Activity:** Incubation of 50 units of enzyme with 1 µg qX174 RF I DNA for 1 hour at 37°C in 50 µl reaction buffer resulted in < 10% conversion to RF II.

**Heat Inactivation:** 70°C for 20 minutes.

**Note:** RNase I, will not degrade DNA. It has a strong preference for single-stranded RNA over double-stranded RNA.

**Applications:**
- Degradation of single-stranded RNA to mono-, di- and trinucleotides (3)
- Used in ribonuclease protection assays

**Reagents Supplied with Enzyme:**
- 1X NEBuffer 3

**Reaction Conditions:**
- Incubate at 37°C.

**Quality Control Assays**

**ss DNA Exonuclease Activity:** Incubation of 50 units of enzyme with 1 µg sonicated and denatured [3H] DNA (10^5 cpm/µg) for 30 minutes at 37°C in 50 µl reaction buffer released < 1% radioactivity.

**ds DNA Exonuclease Activity:** Incubation of 50 units of enzyme with 1 µg sonicated [3H] DNA (10^5 cpm/µg) for 30 minutes at 37°C in 50 µl reaction buffer released < 1% radioactivity.

**Endonuclease Activity:** Incubation of 50 units of enzyme with 1 µg qX174 RF I DNA for 1 hour at 37°C in 50 µl reaction buffer resulted in < 10% conversion to RF II.

**References:**

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**RNase If**

*Source:* An *E. coli* strain containing a genetic fusion of the RNase I gene (rna) from *E. coli* and the gene coding for maltose-binding protein (MBP)(2)

**Description:** Ribonuclease If (RNase If) is a recombinant protein fusion of RNase I (from *E. coli*) and maltose-binding protein. It has identical activity to RNase I.

**Unit Definition:** One unit is defined as the amount of enzyme required to fully digest 1 picomole of synthetic ssRNA 33-mer in a total reaction volume of 10 µl in 15 minutes in 1X NEBuffer 3 as visualized on a 20% acrylamide gel (40:1 Bis) stained with SYBR Gold®.

**Activity:** Incubation of 50 units of enzyme with 1 µg qX174 RF I DNA for 1 hour at 37°C in 50 µl reaction buffer resulted in < 10% conversion to RF II.

**Heat Inactivation:** 70°C for 20 minutes.

**Note:** RNase I, will not degrade DNA. It has a strong preference for single-stranded RNA over double-stranded RNA.

**Applications:**
- Degradation of single-stranded RNA to mono-, di- and trinucleotides (3)
- Used in ribonuclease protection assays

**Reagents Supplied with Enzyme:**
- 1X NEBuffer 3

**Reaction Conditions:**
- Incubate at 37°C.

**Quality Control Assays**

**ss DNA Exonuclease Activity:** Incubation of 50 units of enzyme with 1 µg sonicated and denatured [3H] DNA (10^5 cpm/µg) for 30 minutes at 37°C in 50 µl reaction buffer released < 1% radioactivity.

**ds DNA Exonuclease Activity:** Incubation of 50 units of enzyme with 1 µg sonicated [3H] DNA (10^5 cpm/µg) for 30 minutes at 37°C in 50 µl reaction buffer released < 1% radioactivity.

**Endonuclease Activity:** Incubation of 50 units of enzyme with 1 µg qX174 RF I DNA for 1 hour at 37°C in 50 µl reaction buffer resulted in < 10% conversion to RF II.