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# Murine RNase Inhibitor, GMP-Grade

# Catalog #GMP-RNI-ME101

**Storage Condition**  $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$  for 24 months. Avoid repeated freeze/thaw cycles.

Form Liquid

**Source** *E. Coli* strain that carries the Ribonuclease Inhibitor gene from mouse

**Storage Buffer** 20mM HEPES-KOH, 50mM KCl, 8mM DTT, 50% Glycerol, pH7.6

Concentration 120U/µL

**Unit Definition** One unit is defined as the amount of Murine RNase Inhibitor required to inhibit the activity of 5ng of RNase A by 50%. Activity is measured by the inhibition of hydrolysis of cytidine 2', 3'-cyclic monophosphate by RNase A.

### **Product Contents**

Murine RNase Inhibitor (120U/μL)

# **Product Description**

Murine RNase Inhibitor is a recombinant protein of murine origin that is expressed and purified from *E.coli*. It specifically inhibits RNase A, RNase B, and RNase C by binding noncovalently with RNase in a 1:1 ratio with high affinity. It is not effective against RNase T1, S1 Nuclease, RNase H or RNase from *Aspergillus*. Murine RNase Inhibitor is added to the reactions to improve the stability of RNA and should be added before other components that may contain RNase such as plasmids. Murine RNase inhibitor has no observed inhibition on enzymes used for RNA preparation and analysis.

#### **Applications**

- In vitro transcription
- RT-PCR
- cDNA Synthesis

# **Quality Control Statement**

This product has been filed with the FDA Drug Master Files and is assigned DMF #038031. KACTUS manufactures this product according to GMP guidelines and performs stringent quality control testing before release. The production is antibiotic- and animal-free.

# **Quality Control Release Criteria**

Assay	Criteria
Activity (Hydrolysis Reaction Inhibition)	≥ 120kU/mL
Purity (SEC-HPLC)	≥ 95%
Residual Endonuclease	Negative
Residual Exonuclease	Negative
Endotoxin	≤ 10EU/mL
Residual DNase	Negative
Residual RNase	Negative
Residual Protease	Negative
Residual Host Cell Protein	≤ 20ng/mg
Residual Host Cell DNA	≤ 100pg/mL
Residual Heavy Metal	≤ 10ppm
Bioburden	≤ 1CFU/10mL

## Protocol for In Vitro Transcription

1. Prepare the following reaction mixture.

Reagent	Quantity
5X Transcription Buffer-1 (included with T7 RNA Polymerase)	4µL
CTP/GTP/ATP/UTP (100mM each)	2µL each
Murine RNase Inhibitor (120U/μL)	0.5µL
Pyrophosphatase, Inorganic (0.1U/µL)	1µL
T7 RNA Polymerase (50U/μL)	2µL
Template DNA	1µg
RNase-free Water	Up to 20µL

- 2. Incubate at 37°C for 1-2 hours.
- 3. After transcription, add 2U <u>DNase I</u> to remove DNA template for 15 minutes at 37°C.
- 4. Inactivate DNase I by phenol/chloroform extraction.

#### Notes

- Murine RNase inhibitor can inhibit RNase activity at pH5-9, with highest inhibitory activity at pH7-8.
- Temperatures higher than 50°C or high concentrations of denaturing reagent should be avoided to prevent the release of active RNases from the complex with murine RNase inhibitor.