Murine RNase Inhibitor (40 U/µL)

Product Information

RNase Inhibitor

Catalogue Number	Size
ATR-R604	25 µL (1,000 Units)

Product Description

ATR-MED® RNase Inhibitor is a highly purified, recombinant protein derived from an engineered murine gene sequence, expressed in *Escherichia coli* under animal-component-free conditions. This inhibitor specifically binds to RNase A family enzymes (RNase A, B, and C) with a 1:1 stoichiometry, forming a stable, non-covalent complex that effectively inhibits RNA hydrolysis by sterically blocking the RNase active site. The murine sequence has been genetically modified to eliminate oxidation-sensitive cysteine residues near the active site, significantly enhancing resistance to oxidative inactivation compared to human-derived RNase inhibitors.

Functionally equivalent to leading commercial products such as New England Biolabs® M0314 and Thermo Fisher Scientific® RiboLock™ EO0382, ATR-MED® RNase Inhibitor demonstrates robust performance in the presence of ≥1 mM dithiothreitol (DTT) and maintains activity across a broad range of temperatures (4–50°C) and buffer conditions. It is optimized for high-sensitivity applications, including reverse transcription (RT), real-time quantitative PCR (RT-qPCR), single-cell RNA workflows, RNA sequencing (RNA-seq) library preparation, and *in vitro* transcription/translation (IVT). Each production lot undergoes rigorous quality control to ensure freedom from contaminating RNases, DNases, and proteases, with validated, reproducible inhibitory activity.

Applications

- Reverse transcription (RT) for cDNA synthesis
- Real-time quantitative PCR (RT-qPCR) and one-step qRT-PCR
- RNA sequencing (RNA-seq) library preparation
- *In vitro* transcription and translation systems
- Protection of RNA in single-cell or low-input RNA workflows
- RNA labeling and ligation reactions
- Enzymatic RNA cleanup and purification protocols

Highlights

- **Oxidation-Resistant Design**: Engineered murine sequence eliminates labile cysteine residues, enhancing stability under oxidative conditions.
- **Specific Inhibition**: Binds RNase A, B, and C with high affinity in a 1:1 complex, ensuring robust RNA protection.
- **Robust Activity**: Fully functional in ≥1 mM DTT, compatible with RT, qPCR, IVT, and RNA-seq workflows.
- **High Purity**: Certified free of RNases, DNases, and proteases through stringent quality control.
- **Broad Applicability**: Optimized for high-sensitivity RNA-handling applications, including reverse transcription, RT-qPCR, RNA-seq, and RNA purification.
- **Lot-to-Lot Consistency**: Manufactured under rigorous quality control to ensure reproducible performance.

Source

Recombinantly expressed and purified from an *Escherichia coli* strain harboring the engineered murine RNase inhibitor gene.

Unit Definition

One unit (U) of ATR-MED® RNase Inhibitor is defined as the amount required to inhibit 50% of the activity of 5 ng bovine RNase A in a 30-minute assay at 37°C, pH 7.4, in the presence of 1 mM DTT. At a concentration of 40 U/µL, 1 µL delivers 40 units, aligning with specifications of leading commercial RNase inhibitors (e.g., NEB® M0314, Thermo Fisher® RiboLock™).

Buffer Composition

Storage Buffer: 20 mM HEPES-KOH (pH 7.6 at 25°C), 50 mM KCl, 8 mM DTT, 50% (v/v) glycerol.

Storage

Store at -20°C in a tightly closed container to maintain stability. Avoid repeated freeze-thaw cycles to prevent loss of activity.

Shipping

Shipped on gel ice packs to ensure stability. Upon receipt, transfer immediately to a -20°C freezer.

Inhibition and Inactivation

- **Mechanism of Inhibition**: ATR-MED® RNase Inhibitor forms a tight 1:1 complex with RNase A, B, and C, sterically blocking their active sites to prevent RNA hydrolysis.
- **Denaturant Sensitivity**: The inhibitor-RNase complex is sensitive to denaturation. Avoid:

- Temperatures >50°C.
- High concentrations of denaturants (>1 M urea, guanidine HCl, or SDS).
- Strong oxidizing agents (e.g., p-chloromercuribenzoate, high dissolved oxygen).

- **Heat Inactivation**: Inactivate by heating to 75°C for 10 minutes (residual activity may persist after 70°C for 10 minutes). Use this step to terminate inhibition before downstream applications requiring RNase activity.
- **Recommended Usage**:
  - Final concentration: 1 U/µL in reaction mixtures.
  - Add ATR-MED® RNase Inhibitor before components that may introduce RNases (e.g., plasmid preparations, enzyme stocks).
- **Reductant Requirement**: Active in ≥1 mM DTT, as provided in standard reaction protocols. The storage buffer contains 8 mM DTT for long-term stability; no additional DTT is required in reactions.
- **Contaminant-Free Assurance**: Each lot is certified free of RNases, DNases, and proteases, ensuring RNA degradation occurs only with intentional RNase addition or inhibitor denaturation.

Application-Specific Protocols

1. Reverse Transcription (RT) / RT-qPCR

Reaction Setup (20 µL):

- RNA template: Up to 1 µg
- ATR-MED® RNase Inhibitor: 0.5 µL (20 U)
- Reverse transcriptase, buffer, dNTPs, primers: Per vendor instructions

Procedure:

1. Combine RNA, primers, buffer, and dNTPs in a sterile tube; add ATR-MED® RNase Inhibitor last.
2. Incubate at 25°C for 5 minutes for primer annealing.
3. Perform cDNA synthesis at 42–50°C for 30–60 minutes.
4. Heat-inactivate at 85°C for 5 minutes.
5. Proceed to qPCR or downstream applications.

Note: ATR-MED® RNase Inhibitor ensures RNA integrity; no additional DTT is needed beyond 1 mM.

2. *In Vitro* Transcription (IVT)

Reaction Setup (20 µL):

- DNA template (with T7 promoter): 1 µg
- NTP mix, T7 RNA polymerase, buffer: Per kit instructions
- ATR-MED® RNase Inhibitor: 1 µL (40 U)

Procedure:

1. Assemble DNA, NTPs, buffer, and polymerase; add ATR-MED® RNase Inhibitor.
2. Incubate at 37°C for 2 hours.
3. (Optional) Perform DNase treatment to remove template.
4. Purify RNA using column-based or precipitation methods.

Note: ATR-MED® RNase Inhibitor prevents RNase-mediated RNA degradation during transcription.

3. RNA-Seq Library Preparation

- Include ATR-MED® RNase Inhibitor at 1 U/µL final concentration in all RNA-handling steps (e.g., RNA extraction, fragmentation, adapter ligation).
- Omit RNase inhibitor in final enzymatic steps (e.g., second-strand synthesis) to avoid interference.

Precautions and Disclaimer

This product is designated for research and development purposes only and is not intended for therapeutic, diagnostic, household, or other non-research applications. Handle using standard laboratory protective equipment, including lab coats, disposable gloves, and safety goggles. When using radioactive nucleotides, adhere to institutional radiation safety protocols. Comprehensive safety data are available in the Material Safety Data Sheets (MSDSs) at www.atrmed.com or via email request to info@atrmed.com. To the maximum extent permitted by applicable law, ATR-MED Inc. disclaims liability for special, incidental, indirect, punitive, or consequential damages arising from the use of this product or associated documentation. Product use constitutes acceptance of ATR-MED's terms and conditions. All trademarks are owned by ATR-MED unless otherwise specified.

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