

GenTegra RNAssure™

Immediate and complete protection directly from RNA extraction

Cellular RNA provides data-rich biological information about gene expression. Genomic RNA in viruses is used as the basis for viral genetics. Researchers rely on RNA extraction kits to purify and collect their RNA samples for downstream applications including next-generation sequencing (NGS) RNA-Seq, qPCR, and other expression profiling techniques. Keeping RNA samples on ice all the time can slow RNA degradation. However, during handling, the sample temperature can often rise above 0°C, and even brief exposure to elevated temperature is detrimental to RNA, especially with prevalent contamination from endogenous or environmental RNases^{1,2}. On the other hand, the amount of RNase contamination varies depending on sample types and extraction methods, and it is extremely difficult to assess the level of contamination in an extracted RNA sample. Therefore, it is important to preventively treat the RNA samples with a stabilization agent immediately after extraction to eliminate concerns of RNA degradation during subsequent procedures.

GenTegra RNAssure is a robust, reliable protection product that utilizes GenTegra's proven, patented Active Chemical Protection™ (ACP) chemistry to protect RNA against all RNases (Figure 2) as well as exposure to oxidation. Maximum RNA protection is achieved by treating purified RNA samples with RNAssure as soon as they are isolated. With RNAssure, RNA can be protected even at room temperature (RT), eliminating the concern that RNA may degrade during every day experimental protocols (Figure 3) or due to catastrophic freezer failure. RNAssure can protect RNA for at least 3 days at RT (Figure 4) and 2 weeks at 4°C. GenTegra's RNAssure is easily integrated into extraction and purification kits from all major life science

manufacturers, such as Invitrogen, Zymo, and QIAGEN (Figure 1). RNAssure protected samples can be used directly in downstream applications without the need for further purification.

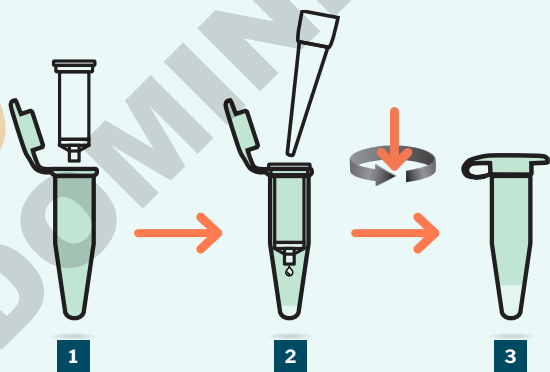
RNAssure also protects RNA during DNase treatment. Column based RNA extraction kits often lead to genomic DNA (gDNA) contamination, comparing to Trizol-based purification methods (Figure 5A), and DNase digestion is commonly performed. However, incubating RNA samples with DNase solution at either RT or 37°C may lead to significant degradation. Utilizing RNAssure during DNase treatment, whether on-column or in-solution digestion, will protect RNA from degradation without compromising the effectiveness of DNase digestion (Figure 5B).

Benefits

- Inactivate all residual RNase activity carried over from purification process or environmental contamination
- Protect RNA from oxidation damage, non-specific adherence to plastic, and adverse or accidental exposure to higher temperatures
- Allow RT handling of RNA samples and 4°C storage for weeks without losing RNA integrity
- Protect RNA during DNase treatment when exposed to RT or 37°C for extended periods of time
- Integrates seamlessly into standard RNA extraction kit workflows

Figure 1: RNAssure Elution Tube simply replaces the manufacturer's elution collection tubes and integrates with standard column-based purification kits and protocols.

1. For the final elution step, simply place the spin column into the RNAssure collection tube. **2.** Add the elution buffer to the column. Centrifuge the column and tube. Remove and discard the column. **3.** Purified RNA is now protected by RNAssure for at least 3 days at RT.



STORAGE CONDITION	LENGTH OF PROTECTION
Room temperature (15 – 25°C)	3 days
Refrigeration (2 – 8°C)	2 weeks
Frozen (< – 70°C)	> 1 year

¹ Precautions for Handling of RNA. https://lifescience.roche.com/en_us/articles/precautions-for-handling-of-rna.html

² The Basics: RNase Control. <https://www.thermofisher.com/us/en/home/references/ambion-tech-support/nuclease-enzymes/general-articles/the-basics-rnase-control.html>

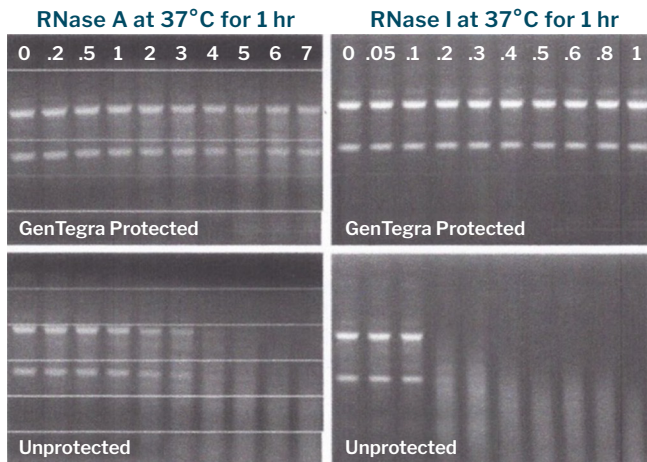


Figure 2: GenTegra RNAssure protects RNA in the liquid state at 37°C in the presence of trace RNase. HeLa cell RNA (5 µg) was incubated with the indicated amounts of RNase at 37°C for one hour in the presence (top row) or absence (bottom row) of GenTegra RNAssure. RNA integrity is maintained in the liquid state in the presence of increasing amounts of RNase A (left) and RNase I (right) only when protected with GenTegra RNAssure.

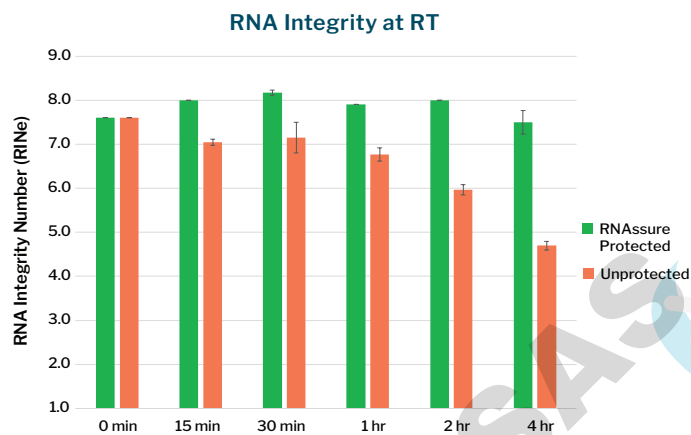


Figure 3: RNAssure protects RNA from degradation during short exposures to RT. Following short incubation at RT, purified mouse spleen RNA samples (commercially sourced) were analyzed on a Bioanalyzer for RIN scores. In the absence of RNAssure (orange), the RNA sample exhibited progressive degradation, starting from 15 min exposure to RT. Whereas in the presence of RNAssure (green), the RNA sample was protected with no significant change in RIN value, throughout the 4 hr exposure to RT.

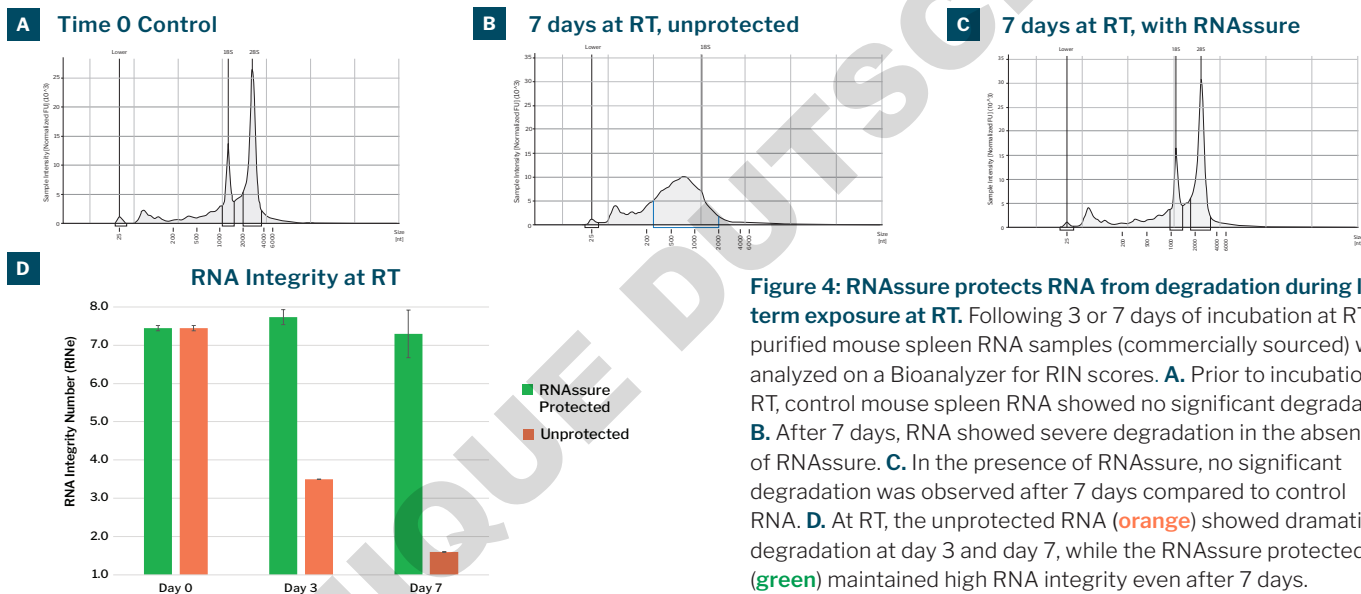


Figure 4: RNAssure protects RNA from degradation during long-term exposure at RT. Following 3 or 7 days of incubation at RT, purified mouse spleen RNA samples (commercially sourced) were analyzed on a Bioanalyzer for RIN scores. **A.** Prior to incubation at RT, control mouse spleen RNA showed no significant degradation. **B.** After 7 days, RNA showed severe degradation in the absence of RNAssure. **C.** In the presence of RNAssure, no significant degradation was observed after 7 days compared to control RNA. **D.** At RT, the unprotected RNA (orange) showed dramatic degradation at day 3 and day 7, while the RNAssure protected RNA (green) maintained high RNA integrity even after 7 days.

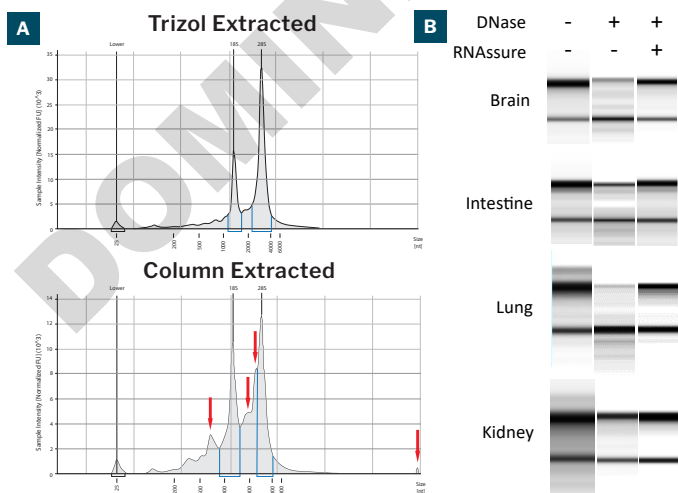


Figure 5: RNAssure protects RNA during DNase Treatment. RNA was extracted from various mouse organs using either Trizol-based method or a leading commercial column-based kit **A.** While mouse liver RNA extracted by Trizol was free of gDNA contamination, column-based kit led to significant contamination of gDNA of various sizes (red arrows). **B.** RNA extracted from brain, intestine, lung, and kidney, using column-based method, was subjected to in-solution DNase treatment following manufacturer's instruction (37°C for 30 min). After treatment, RNA from different samples showed moderate to severe degradation, evidenced by decreased 18S rRNA (top band) vs 28S rRNA (bottom band). In contrast, RNAssure helped significantly to maintain the RNA integrity during DNase digestion.