

ClearBand RNAintact, RNA stabilization solution

Troubleshooting Guide

100 mL

Cat No: R100

Use this guide for common problems encountered during RNA stabilization, sample storage, and recovery of tissue, cultured cells, white blood cells, and bacteria from ClearBand RNAintact. For best results, follow the specified sample size, reagent volume, storage temperature, centrifugation, and recovery conditions in the product protocol.

Before You Start - Quick Checks

1. If precipitates are present in RNAintact, warm to about 40°C and agitate until fully dissolved; cool to room temperature before use.
2. For animal or plant tissue, keep at least one tissue dimension below 0.5 cm and use at least 10 volumes of RNAintact.
3. Completely submerge tissue. For plant tissues with diffusion barriers, disrupt the tissue sufficiently to permit penetration.
4. For cultured cells and bacteria, remove residual medium and add 5-10 equivalent volumes of RNAintact; mix gently by inversion.
5. Observe documented storage limits: up to 1 day at 37°C, 1 week at 25°C, or 1 month at 4°C.
6. For long-term -20°C or -80°C storage, use the specific overnight 2-8°C pre-incubation and reagent-retention/removal procedure described in the datasheet.
7. During recovery, remove residual RNAintact and transfer/homogenize the sample in RNA-isolation lysis solution without unnecessary delay.

Problem	Most likely cause	Recommended action
Precipitates are visible in RNAintact before use	Precipitation can occur during storage, particularly after exposure to lower temperatures.	Warm RNAintact to about 40°C in a water bath and agitate until the precipitates completely disappear. Re-cool the solution to room temperature before use.
Poor RNA stabilization in tissue samples	Tissue pieces are too large, the sample is not fully submerged, or the amount of RNAintact is insufficient for rapid penetration.	Cut animal or plant tissue into small pieces with at least one dimension below 0.5 cm. Use at least 10 volumes of RNAintact and make sure the tissue is completely submerged.
Poor stabilization in plant tissue	Natural barriers in some plant tissues restrict diffusion of the stabilization solution.	Carefully disrupt tissues that have natural diffusion barriers before immersion so RNAintact can penetrate the sample efficiently. Keep the material completely submerged.
Cells are not fully exposed to RNAintact	Residual culture medium remains, or too little RNAintact was added to the pellet.	Pellet tissue-culture cells, wash twice with PBS to remove culture medium, then add 5-10 equivalent volumes of RNAintact. Mix gently by inverting the tube 5-10 times.
Bacterial samples show inconsistent stabilization	Residual culture medium remains or the bacterial pellet is not adequately mixed with RNAintact.	Pellet bacteria, carefully remove remnants of medium, add 5-10 equivalent volumes of RNAintact, and mix gently by inverting 5-10 times.
White blood cell samples are difficult to stabilize consistently	White blood cells were not first isolated from whole blood, or residual red-cell lysis material remains.	Collect white blood cells using ClearBand 10x Red Blood Cell Lysis Buffer (RBCLB-10x), then handle the recovered cells in the same way as tissue-culture cells.
Sample was stored too long at room temperature	Storage duration exceeded the conditions specified for RNAintact-preserved samples.	Use the documented storage limits: up to 1 day at 37°C, 1 week at 25°C, or 1 month at 4°C. For longer storage, follow the -20°C or -80°C procedures.
Long-term storage at -20°C was prepared incorrectly	The sample was transferred directly to -20°C without the specified pre-incubation at 2-8°C.	For -20°C storage, incubate samples in RNAintact at 2-8°C overnight, then transfer the sample together with the reagent to -20°C.
Precipitates appear during -20°C sample storage	RNAintact may form precipitates under the specified -20°C storage condition.	This is expected according to the product protocol and does not affect downstream processing. Follow the normal recovery procedure for the sample.
Long-term storage at -80°C gives inconsistent recovery	Tissue was frozen with RNAintact instead of following the specified reagent-removal step.	For tissue storage at -80°C, incubate the sample in RNAintact at 2-8°C overnight, then transfer the tissue without the reagent to -80°C. Cell pellets may be frozen at -80°C within RNAintact.
Concern that thawing a frozen cell pellet will reduce RNA quality	The cell pellet was stored frozen within RNAintact and must be thawed before recovery.	Thawing frozen cell pellets within RNAintact at room temperature is stated not to affect RNA yield or integrity. Continue with the recommended recovery procedure.
Tissue RNA yield or integrity is poor after recovery	Tissue remained outside stabilization or lysis solution during transfer, or homogenization was delayed after transfer to the RNA-isolation lysis solution.	Remove tissue carefully with sterile forceps, transfer it directly into the RNA-isolation lysis solution, and homogenize immediately.
Tissue requires trimming after storage	The stored tissue piece is still larger than desired for downstream processing.	Remove tissue from RNAintact, cut it into smaller pieces, and immerse it again in RNAintact if desired before continuing.

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Problem	Most likely cause	Recommended action
Tissue-culture cells do not pellet during recovery	RNAintact is a dense solution, so centrifugation speeds normally used for live cells may be insufficient.	Pellet cells using a centrifugal force appropriate for the cell type; 3 minutes at 3000-10000 x g is recommended. Remove residual RNAintact and immediately homogenize the cells in RNA-isolation lysis solution.
Cell pelleting remains difficult in RNAintact	High solution density continues to interfere with efficient pelleting.	Add 3 volumes of 1x PBS to the RNAintact solution to facilitate pelleting, then centrifuge as appropriate for the cell type.
Bacteria do not pellet during recovery	Centrifugation conditions suitable for bacteria in culture medium may be insufficient in dense RNAintact.	Centrifuge bacteria at an appropriate force; 3 minutes at 10000 x g is recommended. Remove residual RNAintact and immediately homogenize bacteria in RNA-isolation lysis solution.
Bacterial pelleting remains difficult	The density of RNAintact makes recovery by centrifugation inefficient.	Add 3 volumes of 1x PBS to the RNAintact solution to help pelleting, then centrifuge at the recommended centrifugal force.
Residual RNAintact is carried into downstream RNA isolation	The stabilization solution was not adequately removed after pelleting cells or bacteria.	After pelleting, remove the remaining RNAintact before homogenizing the sample in the RNA-isolation lysis solution. For tissue, remove the tissue from RNAintact before transfer to lysis solution.

Still having trouble? Re-check sample dimensions and RNAintact volume, full sample immersion, the applicable storage condition, and the recovery centrifugation step. For cells or bacteria that are difficult to pellet, use the recommended centrifugal force and consider adding 3 volumes of 1x PBS. If the problem persists, contact EcoTech Biotechnology technical support with the sample type, sample amount, RNAintact volume, storage temperature/duration, and recovery conditions.