diagenode

A Hologic Company

RNA Extraction kit

Cat. No. C20000010 (AL-001-0050)

USER GUIDE

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Please read this manual carefully
before starting your experiment

Contents

Principle

Isolation of intact RNA is essential for many techniques used in gene expression analysis (eg. RT-PCR). The RNA extraction kit enables the total RNA extraction from tissues. The RNA extraction kit outperforms when used in combination with RNA extraction beads (included in RNA extraction kit) and Bioruptor® sonication. Efficient disruption and homogenization of the starting material is an absolute requirement for all total RNA purification procedures. Disruption and homogenization of tissue samples can be carried out rapidly and efficiently using the Bioruptor®.

As sonication medium maintains the integrity of RNA, while disrupting cells and dissolving cell components. TRIzol is added to a sample, and after dissolving and/or homogenizing using Bioruptor®, chloroform is added to the mixture and centrifuged. The homogenate is separated into 3 phases: an aqueous phase, an organic phase and an intermediate phase. RNA is present in the aqueous phase. The aqueous phase is collected, and RNA is precipitated by adding isopropanol.

Features:

- Validated protocol using the RNA extraction beads and Bioruptor®
- DNA contamination is minimal, and the extracted RNA can be used for RT-PCR and quantitative RT-PCR as it is.
- High quality RNA can be extracted in about one hour.

Kit content

Description	Format	Storage
RNA extraction tubes	50 pc	Room temperature
RNA extraction beads	20 g	Room temperature

RNA isolation

When isolating RNA using TRIzol, intact high quality total RNA can be isolated in about one hour at a high yield. The RNA thus obtained contains almost no DNA or protein, and can be used as-is for Northern analysis, dot blot hybridization, RT-PCR, RT-qPCR.

General remarks before starting

- Minimize the time of tissue collection to prevent RNA degradation by RNases and from changes in RNA expression triggered by sample manipulation. RNA quality correlates to tissue-specific response to physiological stress both prior to and following death.
- Dissected tissues can be snap-frozen in liquid nitrogen and stored at -80°C until RNA extraction. Alternatively, RNAlater solution can be used to protect RNA in unfrozen sample.
- This protocol has been validated for quantities of tissue from 20 to 50 mg. Do not use more tissue per sample as it might result in low quality RNA. For larger quantity, cut the tissue and proceed to disruption in separate tubes.
- When working with TRIzol, work in fume hood and use gloves and eye protection.
- When working with RNA, care must be taken to maintain an RNase-free environment starting with RNA purification and continuing through analysis. Wear gloves at all times to prevent RNase contamination from the surface of the skin or from dusty laboratory equipment. Change gloves frequently and keep tubes closed whenever possible. Keep isolated RNA on ice when aliquots are pipetted for downstream applications.

Required materials and reagents

- Bioruptor® Standard or Plus (Diagenode, Cat. No. UCD-200, UCD-300) for tissue disruption and homogenization
- RNA extraction kit (Diagenode, Cat. No. C20000010)
- Bioruptor® Water Cooler (Diagenode, Cat. No. B02010002, B02010003)
- Single Cycle Valve for Bioruptor® Plus (Diagenode, Cat. No. B02020005)
- Tube holder pack for extraction kits (Diagenode, Cat No. B01200016)
- Liquid nitrogen or RNAlater solution (Ambion, Cat. No. 7020) for tissue collection
- Isopropanol (Molecular Biology grade)
- TRIzol (Molecular Biology grade)
- Chloroform (Molecular Biology grade)
- 70 % ethanol (Molecular Biology grade)
- RNase-free water
- 2 ml RNase-free tubes
- Nanodrop and Agilent Bioanalyzer for quality assessment (optional)

Standard protocol for RNA isolation from tissues using TRIzol, Bioruptor® and RNA extraction beads

Tissue disruption and homogenization using Bioruptor®

1. Pre-cool Bioruptor® to 4°C using the Bioruptor® Water Cooler or crushed ice.

2. Prepare sonication tubes: add 30-40 beads (~ 300 mg) and 1 ml of cold TRIzol to the RNA extraction tubes.

   ***NOTE**: We highly recommend to use these tubes without reflecting bars. Do not use TRIzol if discoloration in the product is observed.*

3. Keep the tubes on ice.

4. Add 20-50 mg of snap-frozen tissue per tube containing TRIzol. Alternatively, RNAlater-treated samples can be used.

   ***NOTE**: If needed, cut the frozen tissue in a Petri dish placed on dry ice. Minimize the time required for this, and do not allow sample to thaw before immersing into TRIzol.*

5. Adjust the sample volume with TRIzol to final volume 2 ml and vortex vigorously.

6. Insert the aluminium rings to ensure an optimal position of the tube in the tube holder during sonication (see picture). To guarantee homogeneity of sonication, the tube holders should always be completely filled with tubes.

7. Sonicate samples in Bioruptor® using the following settings:

   - Power: H position (High)
   - Sonication cycle: 30 seconds OFF, 30 seconds OFF
   - Temperature: 4°C
   - Total sonication time: 1-3 cycles

8. Stop Bioruptor® after each cycle, vortex samples and visually check the sample for disruption.

   ***NOTE**: Please note that optimization might be required depending on sample format (fresh or frozen tissue), tissue type and tissue amount. The shortest sonication time should be chosen to prevent RNA degradation. Incomplete disruption may occur with fibrous tissues like muscles. Do not sonicate longer than 3 cycles to prevent low quality RNA.*

9. Vortex tubes vigorously after sonication and incubate for 5 min at room temperature.

RNA isolation

10. Centrifuge samples at 3.000 rpm for 5 min at room temperature and transfer the supernatant to a new 2 ml RNase-free tube.

    ***NOTE**: This step permits the complete dissociation of nucleoproteins complex.*

11. Add 0.4 ml of chloroform, vortex and centrifuge at 12.000 g for 10 min at 4°C.

    ***NOTE**: Chloroform mixed with isoamyl alcohol should not be used. Thorough mixing is important for subsequent phase separation.*

12. Transfer the colorless upper phase to a new 2 ml tubes. Take care not to aspirate the DNA-containing white interface and organic pink phase.

    ***NOTE**: If contamination of genomic DNA is expected, extract again by adding an equal volume of chloroform to the aqueous phase transferred to the new tube.*

13. Add 0.8 ml of isopropanol, mix and centrifuge at 12.000 g for 10 min at 4°C. A gel-like pellet forms on the side and bottom of the tube.

14. Remove the supernatant and keep the pellet.

15. Add 1 ml of 70% ethanol, vortex samples and centrifuge for 10 min at 12.000 g.

16. Remove the supernatant and air-dry the pellet for 5-10 min at room temperature. Do not over-dry the pellet.

17. Add RNase-free water (100-300 µl depending on expected RNA yield), resuspend carefully by pipetting. The solution can be incubated at 55-60 C° for 10 min if the pellet is hard to dissolve.

18. Take an aliquot for quantitation and quality analysis. Store RNA at -80°C.

RNA quantitation and quality assessment

19. Quantify RNA using a Nanodrop and analyze ratio OD 260/280 and OD 260/230 to ensure the purity of RNA.

    ***NOTE**: Ratio OD260/280 1.8-2.0 is considered good. A low ratio might indicate protein contamination. A ratio greater than 2.1 might indicate RNA degradation.*

    ***NOTE**: Ratio OD260/230 greater than 1.8 is considered good. A low value might indicate organic contamination.*

20. Assess the integrity of RNA using the Agilent 2100 Bioananlyzer (or BioRad Experion system). RIN values threshold depends on the desired downstream experiments and should be correlated with the specific assay to be run (RT-PCR or microarray, for example).

The table below shows suggested applications for RNA within different RIN ranges:

RIN Value	RNA quality	Suggested application
1-4	degraded to low	PCR assays with short regions of amplification
4.1-6.9	moderate to regular	qRT-PCR applications
7.0-10.0	excellent to outstanding	Highly demanding gene array assays

For use in PCR and qRT-PCR, treatment with DNase I (RNase-free) is recommended (see the protocol in the troubleshooting guide).

Example of tissue disruption

Figure 1. An example of mouse brain disruption using the Bioruptor® in combination with the RNA extraction kit.

Left picture shows samples before sonication. Right picture shows disrupted sample.

Examples of total RNA profiles obtained from animal tissues

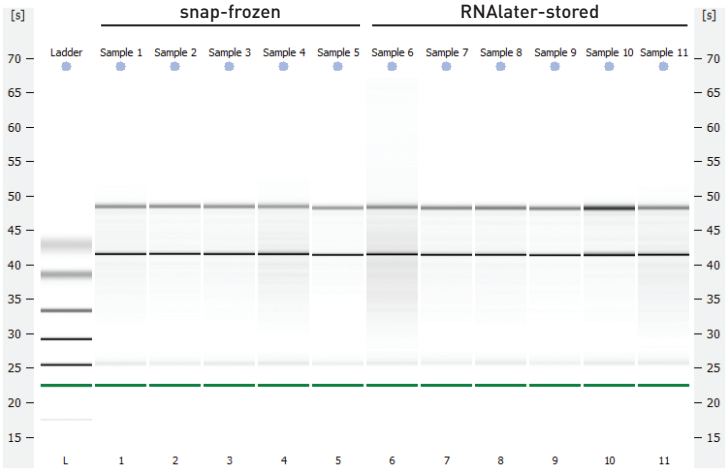


Figure 2. Total RNA efficiently extracted from snap-frozen (lanes 1-5) and RNAlater-treated (lanes 6-11) mouse brain samples.

Tissue was disrupted with the Bioruptor® Standard (UCD-200). Total RNA was extracted as directed in the protocol and analyzed on BioAnalyzer (Agilent).

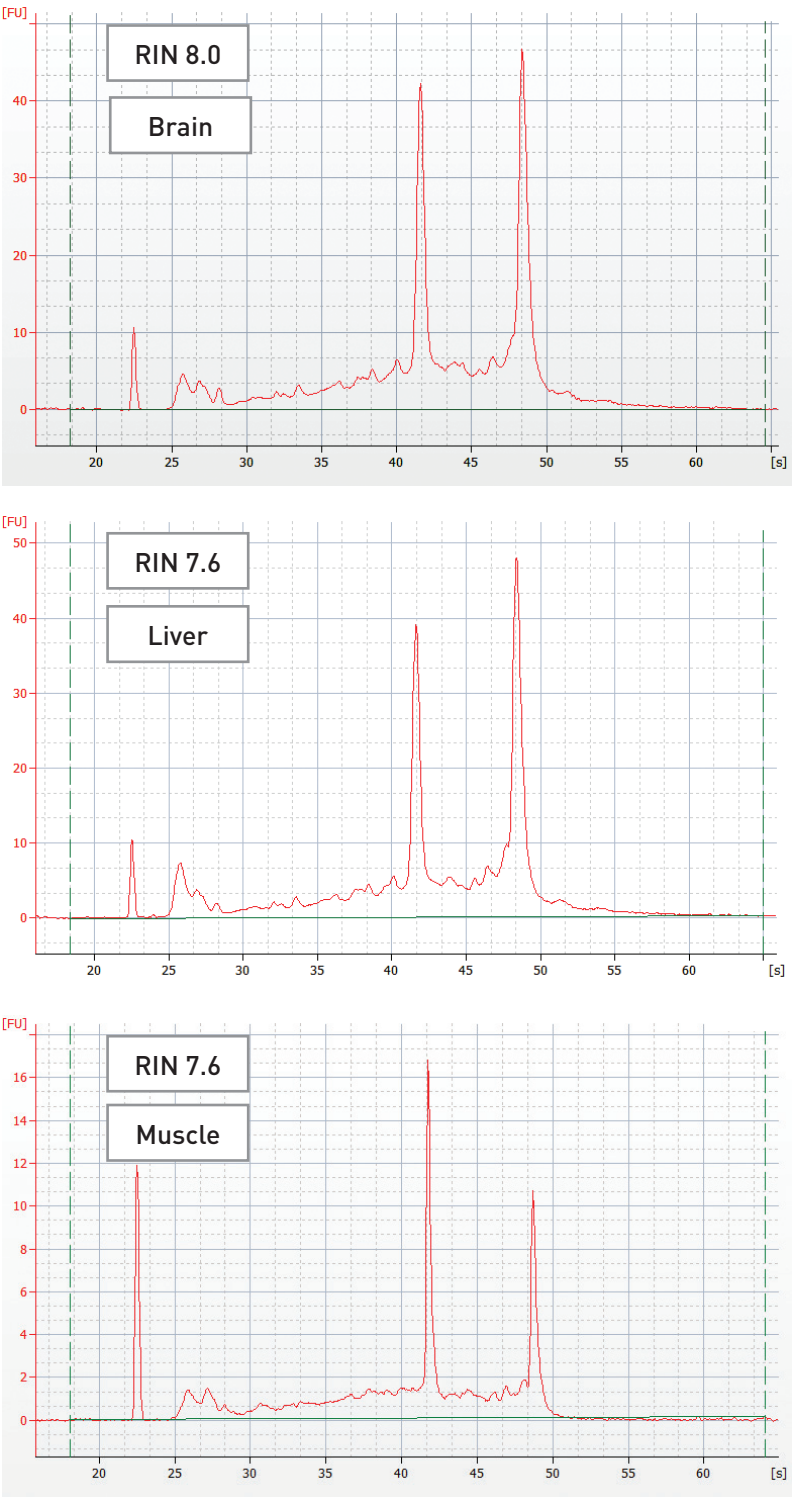


Figure 3. Efficient extraction of pure RNA with high RIN.

Total RNA profiles from mouse brain (upper panel), liver (middle panel) and skeletal muscle (bottom panel). Tissues were disrupted with Bioruptor® Plus (UCD-300) as described in the protocol and analyzed on BioAnalyzer (Agilent). Note that small RNAs are present in all profiles indicating that the RNA is largely intact.

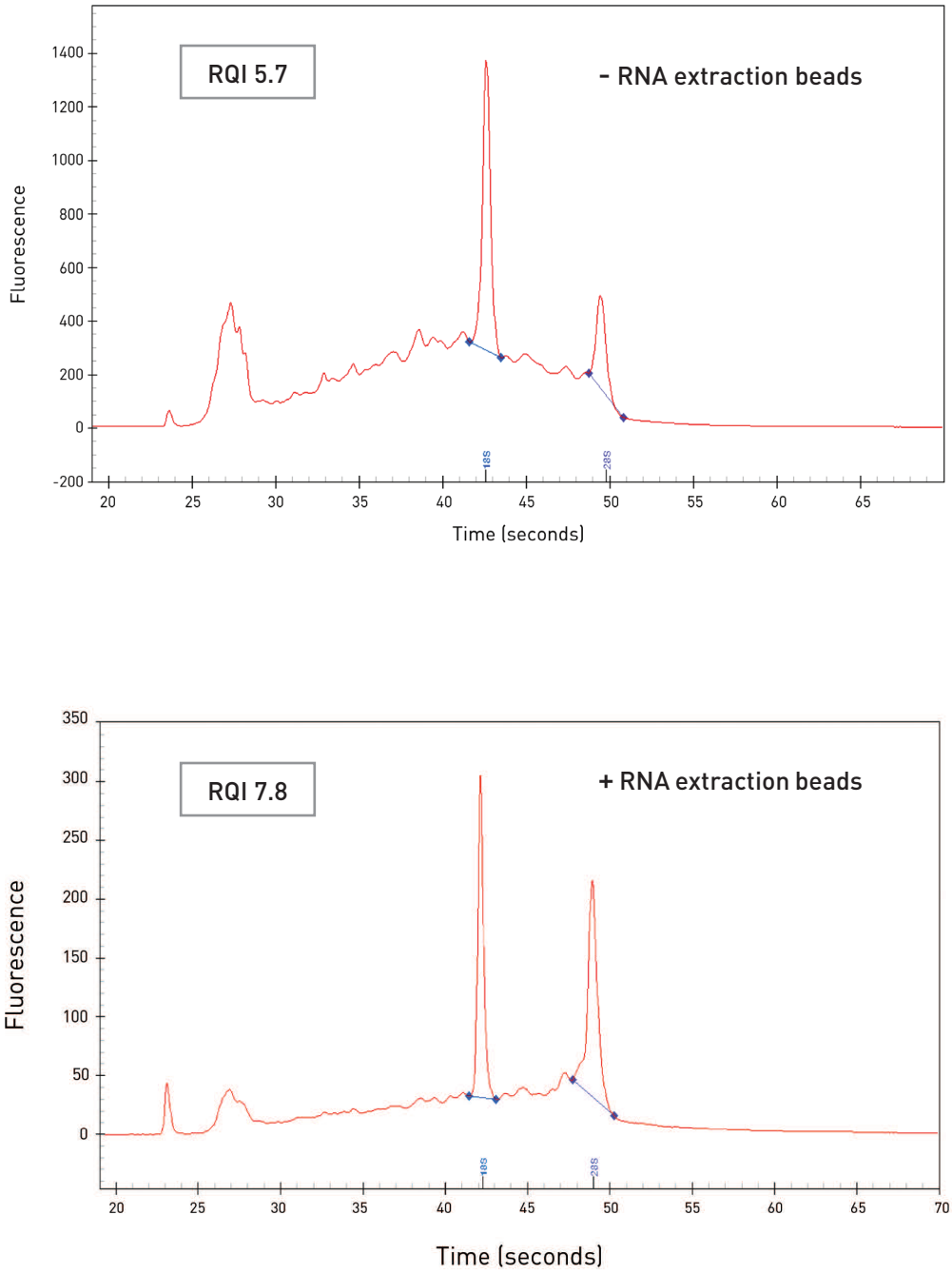
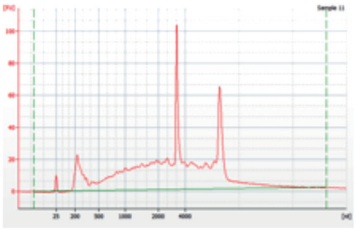


Figure 4. RNA extraction tubes and beads improve tissue disruption and RNA quality.

Experion (BioRad) traces of total RNA obtained from mouse liver using Bioruptor® and the RNA extraction kit without (upper panel) or with RNA extraction beads (bottom panel). Note that only 2 cycles are required for complete tissue disruption using RNA extraction beads vs 15 cycles without RNA extraction beads. RNA extracted from a sample disrupted in the presence of RNA extraction beads shows significantly higher RQI.

Troubleshooting guide

Problem	Possible cause	Suggested solution
Low yields Expected yield of RNA per mg of tissue: • *Liver 4-10 µg* • *Skeletal muscle 0.3-1 µg* • *Brain 2-5 µg* • HeLa cells 6-15 µg per 1X10^6 cells	RNA is not solubilized completely	Do not allow pellet to dry completely. Do not lyophilize or vacuum dry sample.
Degraded RNA	Sample manipulated too much before freezing or RNAlater stabilization Improper storage of RNA Frozen tissue thawed in absence of TRIzol Sample sonicated too much	Process tissue immediately after dissection Store RNA at -80°C avoid thaw-freeze cycles Add frozen tissue immediately to TRIzol Avoid long sonication.1-2 cycles are enough. Do not sonicate longer even if some particulate matter remains
Low RIN value due to high baseline 	DNA/protein contamination is possible	Be sure not to take any of the interphase (contains DNA) with the aqueous phase Do not use more than 50 mg for 2 ml of TRIzol. Remove any particulate material before chloroform addition Treat with RNase-free DNase I (see additional protocol)
Low ratio OD 260/280	Protein contamination	Too much tissue used. Do not use more than 50 mg for 2 ml of TRIzol. Be sure not to carry any organic phase with the RNA sample (step 9 in the protocol)
Low ratio OD 260/230	Organic contamination (chloroform, polysaccharides etc)	Too much tissue used. Do not use more than 50 mg for 2 ml of TRIzol. Be sure not to carry any organic phase with the RNA sample (step 9 in the protocol)

RNA contains some DNA	Part of the interphase was removed with the aqueous phase Too much tissue used for 2 ml of TRIzol Insoluble material was not removed before extraction	Be sure not to take any of the interphase (contains DNA) with the aqueous phase Do not use more than 50 mg for 2 ml of TRIzol. Remove any particulate material before chloroform addition For use in PCR and qRT-PCR, treatment with DNase I (RNase-free) is recommended

Additional protocol for DNase I treatment

- Combine up to 10 µg of RNA, 1 µl of RNase-free DNase (1 U/µl), 5 µl of 10X DNase buffer, 1 µl of RNasin (optional) and RNase-free water to final volume of 50 µl.
- Incubate sample for 15-30 min at room temperature.
- Add EDTA to final concentration 2 mM.
- Extract RNA samples with 100 µl of TRIzol and 20 µl of chloroform. Use 80 µl of isopropanol to precipitate RNA.
- Wash the pellet once with 70% ethanol, air-dry and resuspend in RNase-free water.

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