QIAGEN Supplementary Protocol

Purification of total RNA from tissues and cells using the BioSprint® 96 One-For-All Vet Kit with additional DNase I treatment

This protocol describes purification of total RNA from up to 10 mg animal tissue or 2 x 10^6 cells using the BioSprint 96 One-For-All Vet Kit with additional DNase I treatment, in combination with the BioSprint 96 instrument.

IMPORTANT: Please read the "Safety Information" and "Important Notes" sections in the *BioSprint 96 One-For-All Vet Handbook* and *RNA*later®*-Handbook* before beginning this procedure. For safety information on the additional chemicals mentioned in this protocol, please consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

Equipment and reagents

- BioSprint 96, cat. no. 9000852
- "BS96 toRNA300" protocol, available from QIAGEN Technical Services or your local distributor
- Ordering Code BS96RNA can be used to order the following plasticware and chemistries for purification of total RNA from 384 samples:
  1 x BioSprint 96 One-For-All Vet Kit (384), cat. no. 947057
  Note: Buffer AL, carrier RNA, and Buffer AVE are not required for this protocol.

  4 x RNase-Free DNase Set (50), cat. no. 79254
  2 x RDD Buffer Set for RNeasy 96, cat. no. 1011132
- RNAse-free water (e.g., Nuclease-Free Water (1000 ml), cat. no. 129115
- Multichannel pipet and disposable pipet tips (10–1000 µl) with aerosol barriers
- TissueLyser II system or TissueRuptor®, or an alternative bead-mill or rotor-stator homogenizer, or a pestle and mortar with QIAshredder column, cat. no. 79654
- Ethanol (96–100%)*
- Vortexer
- 14.3 M β-mercaptoethanol (β-ME)
- Shaker-incubator (e.g., Eppendorf® Thermomixer)
- Tubes for DNA storage
- Soft cloth or tissue and 70% ethanol to clean the worktable

* Do not use denatured alcohol because this contains other substances such as methanol or methylethylketone.

Important points before starting

- Fresh, frozen, or RNA*later* stabilized tissue can be used.
- For long-term storage of frozen tissue, flash-freeze in liquid nitrogen and transfer immediately to –70°C. Tissue can be stored for several months at –70°C. Before disruption in Buffer RLT, do not allow tissue to thaw during weighing or handling.
- Homogenized tissue lysates (in Buffer RLT, step 3) can also be stored at –70°C for several months. To process frozen lysates, thaw samples at room temperature or at 37°C in a water bath until they are completely thawed and salts in the lysis buffer have dissolved. Avoid extended treatment at 37°C. This can cause degradation of the RNA.

Things to do before starting

- Prepare one of the 2 bottles of 121 ml Buffer AW1 and RPE according to the instructions in the *BioSprint 96 One-For-All Vet Handbook*.
- Prepare Rebind Buffer by adding 363 ml absolute ethanol to one bottle of 121 ml Buffer AW1.
  IMPORTANT: Ensure that the bottle containing Rebind Buffer is correctly labeled.
- Prepare DNase I stock solution. Dissolve solid DNase I (1500 Kunitz units) in 1100 µl of the RNase-free water (supplied). To avoid loss of DNase I, inject the RNase-free water into the vial using a syringe and needle. Mix gently by inverting the vial. Do not vortex.
- For long-term storage of reconstituted DNase I, divide into single-use aliquots and store at –70°C for up to 9 months. Thawed aliquots can be stored at 2–8°C for up to 6 weeks. Do not refreeze the aliquots after thawing. Prepare working solution for DNase I treatment, add 190 µl Buffer RDD to 10 µl DNase I solution.
- Buffer RLT may form a precipitate upon storage. If necessary, redissolve in a 37°C water bath and then place at room temperature (15–25°C).
- Add 10 µl β-ME per 1 ml Buffer RLT. Dispense in a fume hood and wear appropriate protective clothing. Buffer RLT can be stored at room temperature for 1 month after addition of β-ME.

Procedure

1. **Excise the tissue sample, or remove it from storage. Use forceps to remove RNA*later* stabilized tissues from the reagent. Do not use more than 10 mg tissue. Proceed immediately with step 2.**

   Note: For tissue with a high cell density (e.g., spleen), do not use more than 5 mg.

2. **For RNA*later* stabilized tissues, proceed to step 2a. For unstabilized fresh or frozen tissues, proceed to step 2b.**

2a. RNA*later* stabilized tissues:

If the entire piece of RNA*later* stabilized tissue will be used for RNA purification, place it directly into a suitably sized vessel for disruption and homogenization, and proceed with step 3.

If a portion of the RNA*later* stabilized tissue will be used, place the tissue on a clean surface for cutting, and cut it. Determine the weight of the piece to be used, and place it in a suitably sized vessel for homogenization. Proceed with step 3.

Note: RNA in the RNA*later* stabilized tissue is still protected when the tissue is processed at 15–25°C. This allows tissue to be cut and weighed at ambient temperatures. It is therefore not necessary to cut the tissue on ice, dry ice, or in a refrigerated room. The remaining tissue can be placed into RNA*later* RNA Stabilization Reagent and stored for up to 4 weeks at 2–8°C, up to 7 days at 15–25 °C, or up to 1 day at 37°C. For archival storage at –20°C or –80°C, please refer to the *RNA*later *Handbook*.

2b. Unstabilized fresh or frozen tissues:

If the entire piece of tissue will be used for RNA purification, place it directly into a suitably sized vessel for disruption and homogenization. Proceed immediately with step 3.

If a portion of the tissue will be used, determine the weight of the piece to be used, and place it into a suitably sized vessel for homogenization. Proceed immediately with step 3.

3. There are 3 different methods for tissue disruption and homogenization. To use the TissueRuptor, proceed to step 3a. To use a pestle and mortar, in combination with a QIAshredder column, proceed to step 3b. To use a TissueLyser system, proceed to step 3c.

3a. TissueRuptor homogenization:

Add 300 µl Buffer RLT to the tissue. Homogenize using the TissueRuptor for approximately 20–40 s, until the sample is homogeneous. Proceed with step 4.

Note: Rotor-stator homogenization simultaneously disrupts and homogenizes the sample.

3b. Pestle and mortar, in combination with a QIAshredder column:

Place the tissue in a pestle and mortar with liquid nitrogen. Grind the tissue thoroughly. Allow liquid nitrogen to evaporate, but do not allow the tissue to thaw. Add 300 µl Buffer RLT to the frozen tissue powder. Place a QIAshredder spin column into a 2 ml collection tube. Pipet the lysate directly onto the QIAshreddder spin column. Centrifuge for 2 min at maximum speed. Proceed with step 4.

Note: Grinding the sample using a pestle and mortar will disrupt the sample, but will not homogenize it. Homogenization is carried out by centrifugation through the QIAshredder spin column.

3c. TissueLyser II homogenization:

Place the tissue in a 2 ml microcentrifuge tube (not supplied). Add 300 µl Buffer RLT to the tissue and add one stainless steel bead (3–7 mm diameter). Homogenize the

tissue for 2 min at 20 Hz using a TissueLyser II system. Rotate the TissueLyser rack, and homogenize for another 2 min at 20 Hz. Proceed with step 4.

4. **Cool an RNase-free 2 ml microcentrifuge tube (not supplied) using liquid nitrogen. Transfer the tissue lysate into the microcentrifuge tube. Centrifuge at maximum speed for 3 min. Carefully pipet the supernatant into a single well of an S-Block.**
5. **Add 25 µl MagAttract Suspension G, 300 µl ethanol (96–100%), and 40 µl Proteinase K solution to the lysate in the S-Block.**
   Note: Before use, ensure that MagAttract Suspension G is fully resuspended. Vortex for 3 min before using for the first time, and for 1 min before subsequent uses.
6. **Repeat steps 1–5 for each tissue sample.**
7. **Prepare one 96-well microplate and five S-Blocks according to the table below. In each plate or block, the number of wells to be filled with buffer should match the number of samples to be processed (e.g., if processing 48 samples, fill 48 wells per plate or block). Ensure that buffers are added to the same positions in each plate or block (e.g., if processing 48 samples, fill wells A1–H1 to A6–H6 of each plate or block).**

Slot	Message on LCD display	96-well microplate or S-Block	Liquid to be added	Volume per well (µl)
7	Load Elution Plate	96-well microplate	RNase-free water	200
6	Load 3rd RPE wash	S-Block	Buffer RPE	450
5	Load 2nd RPE wash	S-Block	Buffer RPE	450
4	Load RDD Digestion	S-Block	Buffer RDD	190
			RNase-free DNase	10
3	Load 1st RPE wash	S-Block	Buffer RPE	500
2	Load AW1 Wash	S-Block	Buffer AW1	700
1	Load Sample	S-Block	Lysate	665

8. **Switch the BioSprint 96 instrument on at the power switch.**
9. **Slide open the front door of the protective cover.**
10. **Select the protocol "BS96 toRNA300" using the ^ and ^ keys on the BioSprint 96 instrument. Press "Start" to start the protocol run.**
11. **Place the Rod Cover into the S-Block containing lysates.**
12. **A message, "Load Elution Plate", will appear on the LCD. Load slot 7 of the worktable with the elution plate containing RNAse-free water. After loading slot 7, press "Start".**

13. **The worktable will rotate and a new message will appear, asking you to load slot 6 with the third RPE wash plate. Load slot 6, and press "Start" again. Continue this process of pressing "Start" and loading a particular slot until all slots are loaded.**
    Note: Each slot is labeled with a number. Load each 96-well plate or S-Block so that well A1 is aligned with the label (i.e., well A1 faces inward).
14. **Check that the protective cover is correctly installed. It should fit exactly into the body of the BioSprint 96. Slide the door shut to prevent sample contamination.**
    Note: See the *BioSprint 96 User Manual* for safety information.
15. **Press "Start" to start sample processing.**
16. **After the DNase I digestion step, a beep will sound. The message "Add 700 µl Rebind" will be displayed in the LCD. The S-Block containing the DNAse I digested samples will rotate to the front. Add 700 µl Rebind Buffer to each well containing samples.**
    Note: The number of wells to be filled with Rebind Buffer should match the number of samples to be processed.
17. **When you have added the Rebind Buffer, press "Start" to continue the protocol.**
18. **After the samples have been processed, remove the 96-well microplates and S-Blocks as instructed by the display of the BioSprint 96. Press "Start" after removing each 96-well microplate or S-block. You will be instructed to remove the S-Block that contains the samples first.**
    Note: For short-term storage (i.e., up to 24 hours), store the purified RNA at 2–8°C. For long-term storage (i.e., over 24 hours), store the purified RNA at –20°C.
19. **Press "Stop" when all 96-well microplates and S-Blocks have been removed.**
20. **Discard the used 96-well microplates, S-Blocks, and Rod Cover according to your local safety regulations.**
21. **Switch off the BioSprint 96 instrument at the power switch.**
22. **Wipe the worktable and adjacent surfaces using a soft cloth or tissue moistened with distilled water or detergent solution. If infectious material is spilt on the worktable, clean using 70% ethanol or other suitable disinfectant.**

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

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