User-Developed Protocol:

Isolation of genomic DNA and/or proteins from fatty tissue samples treated with QIAzol™ Lysis Reagent

This protocol has been adapted by customers for the isolation of genomic DNA and/or protein from the interphase and organic (phenol) phase of fatty tissue samples lysed in QIAzol™ Lysis Reagent. **It has not been thoroughly tested and optimized by QIAGEN®.**

Two procedures are given below: Protocol 1 describes the isolation of genomic DNA from the combined interphase and organic phase fractions of QIAzol Reagent-lysed samples; and Protocol 2 describes the isolation of the protein fraction from the interphase–organic phase mixture.

IMPORTANT: Please be sure to read the *RNeasy Lipid Tissue Mini* or *Midi Handbook,* or the *QIAzol Handbook* before beginning this procedure, paying careful attention to the Safety Information section.

Important points before starting

- Unless stated otherwise, all protocol steps should be performed at room temperature (15–25°C).
- This protocol is for use with samples lysed in 1 ml QIAzol Lysis Reagent according to the RNeasy Lipid Tissue Mini Protocol. When processing samples lysed in greater volumes of QIAzol Reagent, the volumes of the other reagents and solutions used in the procedures below should be adjusted accordingly.

Things to do before starting

- For preparation of RNA follow the protocol "Total RNA isolation from Lipid Tissues" in the *RNeasy Lipid Tissue Mini* or *Midi Handbook* until step 9, or the protocol “Lysis and Homogenization of Fatty Tissues“ in the *QIAzol Handbook*, until step 8. Be sure to completely remove the upper aqueous phase. Save the interphase and the organic phase for preparation of genomic DNA and/or proteins.
- Complete the RNA preparation before carrying out the procedures below for isolation of genomic DNA and protein from the interphase and organic phase. If necessary, the interphase and organic phase can be stored at 4°C overnight.

Reagents to be supplied by the user

When working with hazardous chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

For genomic DNA isolation procedure

- 75% ethanol
- 100% ethanol
- 0.1 M sodium citrate solution (0.1 M sodium citrate in 10% ethanol [p.a.])
- 8 mM NaOH
- 0.1 M HEPES (free base)
- 100 mM EDTA

For protein isolation procedure

- 100% ethanol
- Isopropanol
- Guanidine-ethanol solution (0.3 M guanidine-hydrochloride in 95% ethanol)
- Urea/DTT solution (10 M urea, 50 mM DTT in water). Dissolve urea in a small volume of water by stirring; weigh DTT just before use, dissolve in a small volume of water, and add to the urea solution while stirring; add water to adjust the final concentrations.

Protocol 1: Isolation of genomic DNA from the organic phase of QIAzol Reagent-lysed fatty tissue samples

Complete the RNA preparation before carrying out the procedures below for isolation of genomic DNA from the interphase. If necessary, the interphase and phenol phase can be stored at 4°C overnight.

Procedure

1. **Remove any residues of the aqueous phase.**
   This is critical for the quality of the isolated DNA.
2. **Add 0.3 ml of 100% ethanol to the interphase and phenol phase, and carefully mix samples by inversion.**
3. **Incubate samples at room temperature (15–25°C) for 2–3 min.**
4. **Centrifuge at 2000 x *g* for 2 min at 4°C to sediment DNA.**
5. **Remove the phenol/ethanol supernatant and save for subsequent protein isolation.**
   Store the phenol/ethanol supernatant at 4°C, or start from step 5 in protocol 2 to isolate protein immediately.

6. **Add 1 ml sodium citrate solution to the DNA pellet. Incubate at room temperature for 30 min, with mixing by inversion every 5 min.**
7. **Centrifuge at 2000 x *g* for 5 min at 4°C, and remove the supernatant.**
8. **Repeat steps 6 and 7 twice.**

   After this wash step the DNA pellet can be stored in 75% ethanol at 4°C for over 3 months. For storage, remove the sodium citrate solution and add 2 ml 75% ethanol without redissolving the pellet.
9. **Add 2 ml of 75% ethanol to the DNA pellet. Incubate at room temperature for 20 min, with mixing by inversion every 5 min.**
10. **Centrifuge at 2000 x *g* for 5 min at 4°C and completely remove the ethanol supernatant.**

    This ethanol wash removes pink color from the DNA pellet.
11. **Air-dry the DNA pellet for 5–15 min.**

    Do not dry using centrifugation, as the pellet will be more difficult to dissolve.
12. **Redissolve the pellet in 8 mM NaOH to achieve the desired DNA concentration.**

    Typically, addition of 300–600 µl of 8 mM NaOH to DNA isolated from 10^7 cells or 50–70 mg tissue will result in a DNA concentration of 0.2–0.3 µg/µl. Resuspension in a weak base is recommended since isolated genomic DNA does not resuspend well in water or Tris buffers.

    At this stage, the DNA preparation (especially from tissues) may contain insoluble gel-like material (e.g., fragments of membranes).
13. **Centrifuge at 14,000 x *g* for 10 min at room temperature to remove insoluble material, and transfer the supernatant to a new tube.**

    The pH of 8 mM NaOH is approximately 9. For storage, the pH of the DNA sample solution should be adjusted to pH 7–8 by addition of TE or HEPES buffer.
14. **To neutralize the DNA sample add 60 µl 0.1 M HEPES and 5.5 µl 100 mM EDTA (final concentration 1 mM) per 500 µl 8 mM NaOH used for redissolving the DNA pellet in step 13.**

    Once the pH is adjusted, DNA can be stored long term at 4°C or –20°C.

Protocol 2: Isolation of the protein fraction from the organic phase of QIAzol Reagent-treated fatty tissue samples

Complete the RNA preparation before carrying out the procedures below for isolation of protein from the phenol phase. If necessary, the interphase and phenol phase can be stored at 4°C overnight.

Procedure

1. **Remove any residues of the aqueous phase.**
2. **Add 0.3 ml of 100% ethanol to the interphase and phenol phase, and carefully mix samples by inversion.**
3. **Incubate samples at room temperature (15–25°C) for 2–3 min.**
4. **Centrifuge at 2000 x *g* at 4°C for 2 min to sediment DNA.**
5. **Transfer the phenol/ethanol supernatant containing the protein fraction to a new safe-lock reaction tube.**

   The DNA pellet can be washed in sodium citrate and stored in 75% ethanol at 4°C for over 3 months (see protocol 1, step 6–8).
6. **Add 1.5 ml isopropanol to precipitate the protein, and mix by inversion for 15 s.**
7. **Incubate samples at room temperature (15–25°C) for 10 min.**
8. **Centrifuge at 12,000 x *g* for 10 min at 4°C and remove the supernatant.**
9. **Add 2 ml guanidine–ethanol solution to the pellet containing the protein, and incubate at room temperature for 20 min.**

   The protein pellet can be stored in guanidine-ethanol solution at 4°C (for at least 1 month) or –20°C (for at least 1 year).
10. **Centrifuge at 7500 x *g* for 5 min at room temperature, and remove the supernatant.**
11. **Repeat steps 9 and 10 twice.**
12. **Add 2 ml of 100% ethanol to the pellet containing the proteins and vortex. Incubate at room temperature for 20 min.**
13. **Centrifuge at 7500 x *g* for 5 min at room temperature, and remove the supernatant.**
14. **Air-dry the pellet for 5–10 min.**

    Do not dry under centrifugation, as the pellet will be more difficult to dissolve.
15. **Add 50 µl urea/DTT solution, and break up the pellet using a needle.**
16. **Add 450 µl urea/DTT solution, and incubate at room temperature for 1 h.**
17. **Incubate at 95°C for 3 min then place the tube on ice. During the incubation on ice sonicate 10 times using short bursts.**

    All proteins should be in solution. If not, repeat step 17 once or twice. Sonication should be performed on ice to avoid foaming of the sample.
18. **Centrifuge at 10,000 x *g* for 10 min at room temperature. Transfer the supernatant containing the proteins to a new tube.**

    Proteins dissolved in urea/DTT solution can be directly analyzed by SDS-PAGE/Western blot or protein assays such as Bradford, or stored at 4°C overnight or at –20°C for at least 1 year.

QIAzol Lysis Reagent is a subject of US Patent No. 5,346,994 and foreign equivalents.

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Selected kit handbooks can be downloaded from www.qiagen.com/literature/
Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from www.qiagen.com/ts/msds.asp