EMORY UNIVERSITY

Emory Integrated Genomics Core

Emory Integrated Core Facilities

EIGC.002_Appendix N_QIAGEN QIAamp DNA Mini for Tissue

Standard Operating Procedure Staff Review Page:

I have read and understand the procedure listed above.

Employee name	Date SOP review complete

Standard Operating Procedure Approval Page:

Date Implemented:	23 January 2008	
Updated:	Ashima Amin **Name**	15 June 2020 **Date**
Supersedes:	Ashima Amin **Name**	11 April 2016 **Date**

Annual Review and Approval

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Laboratory Director

Changes Made:
061520: Review, Protocols split out into appendixes. Renumbered SOPs. Previously EIGC.003.

Changes to previous procedures:
041116: Review, LIMS steps updated

Introduction

This protocol is for purification of total (genomic, mitochondrial, and viral) DNA from tissue using a microcentrifuge.

QIAamp DNA Mini Kit provides fast and easy methods for purification of total DNA for reliable PCR and Southern blotting. Total DNA (e.g., genomic, viral, mitochondrial) can be purified from a variety of specimen types, including cultured cells.

DNA is purified using a QIAamp spin column, yielding DNA ready for direct amplification or downstream application. The procedure requires no phenol/chloroform extraction or alcohol precipitation and involves very little handling. DNA is eluted in Buffer AE or water. DNA purified using QIAamp Kits is up to 50 kb in size, with fragments of approximately 20–30 kb predominating. DNA of this length denatures completely during thermal cycling and can be amplified with high efficiency. Purified DNA can be stored at 4°C for immediate use or can be safely stored at –20°C for later use. The purified DNA is free of protein, nucleases, and other contaminants or inhibitors.

Kit Contents

QIAamp DNA Kits	Blood Mini (50)	Blood Mini (250)	Mini (50)	Mini (250)
Catalog no.	51104	51106	51304	51306
Number of preps	50	250	50	250
QIAamp Mini Spin Columns	50	250	50	250
Collection Tubes (2 ml)	150	750	150	750
Buffer AL[a]	12 ml	2 x 33 ml	12 ml	2 x 33 ml
Buffer ATL	–	–	14 ml	50 ml
Buffer AW1[a] **(concentrate)**	19 ml	98 ml	19 ml	98 ml
Buffer AW2[b] **(concentrate)**	13 ml	66 ml	13 ml	66 ml
Buffer AE	15 ml	60 ml	2 x 15 ml	128 ml
QIAGEN® Protease[e]	1 vial[c]	1 vial[d]	–	–
Protease Solvent[b]	1.2 ml	5.5 ml	–	–
Proteinase K[e]	–	–	1.25 ml	6 ml

[a] Contains chaotropic salt. Not compatible with disinfecting agents containing bleach; see handbook for safety information.

[b] Contains sodium azide as a preservative.

[c] Resuspension volume 1.2 ml.

[d] Resuspension volume 5.5 ml.

[e] QIAGEN® Protease and Proteinase K are stored at 4°C.

Procedure

Notes before starting

- Use tissue which has not been previously thawed, if possible. Avoid repeated freezing and thawing of stored tissue samples, since this leads to reduced DNA size.
- Use carrier DNA if the sample contains <10,000 genome equivalents. See handbook for details.
- Heat a thermal shaker to 56°C, and heat a heat block to 70°C.
- All centrifugation steps are carried out at room temperature (15–25°C).
- If a precipitate has formed in Buffer ATL or Buffer AL, dissolve by incubating at 56°C.
- Ensure that Buffer AW1 and Buffer AW2 have been prepared according to the instructions as indicated on the bottle or the table above.
- You will need the following additional equipment and reagents:
    - Phosphate buffered saline (PBS)
    - Microcentrifuge set to room temperature
    - Stainless Steel Beads, 5 mm (Cat No. 69989)
    - TissueLyser

1. **Determine the amount of tissue for each sample. If tissue mass was not provided on the manifest, keeping the samples frozen, use a scale to weigh all of the samples (alternatively, determine the weights of a subset of the samples and estimate the remainder). Do not use more than 25 mg of tissue.**

   The tissue may be more easily handled if kept on dry ice.
   If DNA is prepared from spleen tissue, no more than 10 mg should be used.
   25 mg of tissue will yield approximately 5-30 µg of DNA.

2. **Place a 5 mm stainless steel bead inside a 2 ml screw cap microcentrifuge tube. Add up to 25 mg of tissue (10 mg of spleen) to the tube with 80 µl PBS.**

   Note: Some tissue types may not lyse if ATL lysis buffer is diluted with PBS. In this case, samples will need to be prepared by grinding in liquid nitrogen and then suspending tissue directly in undiluted Buffer ATL for complete lysis.

3. **Balance the tubes in the TissueLyser Adapter Set. Operate the TissueLyser for 20 s at 30 Hz.**

4. **Add 100 µl Buffer ATL.**

5. **Add 20 µl proteinase K, mix by vortexing, and incubate at 56°C with shaking until the tissue is completely lysed. Vortex occasionally during incubation to disperse the sample.**

   Note: Proteinase K must be used. QIAGEN Protease has reduced activity in the presence of Buffer ATL.

   Lysis time varies depending on the type of tissue processed. Lysis is usually complete in 1–3 h. Lysis overnight is possible and does not influence the preparation. Lysis is complete when the

sample no long has visible tissue chunks. To ensure efficient lysis, a shaking water bath or a rocking platform should be used. If not available, vortexing 2–3 times per hour during incubation is recommended.

6. **Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid. Add 4 µl RNase A (100 mg/ml), mix by pulse-vortexing for 15 s, and incubate for 2 min at room temperature (15–25°C).**

7. **Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from inside the lid. Add 200 µl Buffer AL to the sample. Mix again by pulse-vortexing for 15 s and incubate at 70°C for 10 min. Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from inside the lid.**

   It is essential that the sample and Buffer AL are mixed thoroughly to yield a homogeneous solution.

   A white precipitate may form upon addition of Buffer AL. In most cases it will dissolve during incubation at 70°C. The precipitate does not interfere with the QIAamp procedure or with any subsequent application.

8. **Add 200 µl ethanol (96–100%) to the sample, and mix by pulse-vortexing for 15 s. After mixing, briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from inside the lid.**

   It is essential that the sample, Buffer AL, and the ethanol are mixed thoroughly to yield a homogeneous solution.

   A white precipitate may form on addition of ethanol. It is essential to apply all of the precipitate to the QIAamp Mini spin column. This precipitate does not interfere with the QIAamp procedure or with any subsequent application.

9. **Carefully apply the mixture from step 8 (including the precipitate) to the QIAamp Mini spin column (in a 2 ml collection tube) without wetting the rim. Close the cap, and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp Mini spin column in a clean 2 ml collection tube (provided), and discard the tube containing the filtrate.***

   * Flow-through contains Buffer AL or Buffer AW1 and is therefore not compatible with bleach.

10. **Carefully open the QIAamp Mini spin column and add 500 µl Buffer AW1 without wetting the rim. Close the cap, and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp Mini spin column in a clean 2 ml collection tube (provided) and discard the collection tube containing the filtrate.***

11. **Carefully open the QIAamp Mini spin column and add 500 µl Buffer AW2 without wetting the rim. Close the cap and centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 3 min.**

12. **Place the QIAamp Mini spin column in a new 2 ml collection tube (not provided) and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 min.**

13. **Place the QIAamp Mini spin column in a clean 1.5 ml microcentrifuge tube (not provided) and discard the collection tube containing the filtrate. Carefully open the QIAamp Mini spin column and add 200 µl distilled water. Incubate at room temperature (15–25°C) for 5 min and then centrifuge at 6000 x g for 1 min.**

14. **Elute a second time using 100 µl of distilled water, for a total of 300 µl.**

    Note: if your sample input is low, you may elute in lower volumes.