EMORY UNIVERSITY

Emory Integrated Genomics Core

Emory Integrated Core Facilities

EIGC.002_Appendix L_QIAGEN QIAamp DNA Mini for Whole Blood, Buffy Coat, PBMC, Plasma, or Serum

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

Michael Zwick, PhD
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 whole blood, buffy coat, PBMC, plasma, or serum 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 whole blood, PBMC, plasma or serum.

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

- Avoid repeated freezing and thawing of stored blood 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 water bath or heating block to 56°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

1. **Determine the sample type and prepare the sample for extraction:**

    a. **For whole blood, buffy coat, plasma, or serum, add 200 µl of the sample to a tube. If the volume of whole blood or buffy coat are limiting, it is possible to mix 100 µl of sample with 100 µl of PBS.**

    b. **Centrifuge up to 5 x 10^6 PBMC at 1200 x g for 2 min. Remove media. Resuspend cell pellet in 200 µL PBS.**

    c. **Centrifuge up to 5 x 10^6 lymphocytes at 1200 x g for 2 min. Remove media. Resuspend cell pellet in 200 µL PBS.**

    200 µl of whole blood will yield approximately 3-12 µg of DNA.

    200 µl of buffy coat will yield approximately 25-50 µg of DNA.

2. **Add 20 µl QIAGEN Protease (or proteinase K).**

3. **Add 4 µl of an RNase A stock solution (100 mg/ml).**

4. **Add 200 µl Buffer AL to the sample. Mix by pulse-vortexing for 15 s.**

    Note: Do not add QIAGEN Protease, proteinase K, or RNase A directly to Buffer AL.

5. **Incubate at 56°C for 10 min.**

6. **Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.**

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

8. **Carefully apply the mixture from step 6 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 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.

   When preparing DNA from buffy coat or lymphocytes, centrifugation at full speed is recommended to avoid clogging.

9. **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 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.***

10. **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) for 3 min.**

11. **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.**

12. **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.**

13. **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.