



<b>ATF-LS-FB35</b> <b>General QIAamp Micro Double AL DNA Extraction</b>	Published Online: <b>March 2018</b>
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Unofficial Copy; May Not Be Most Current Version	Page: 1 of 6

## 1. Scope

This protocol is to be used to extract and purify DNA from reference samples and samples of evidence containing biological material. DNA can be extracted from dried biological stains or liquid biological material efficiently using the double Buffer AL modification of the QIAamp DNA Micro Kit extraction protocol described below. The method is composed of four basic steps. First, the biological material is digested and the cells lysed in a buffer containing a detergent and a protease. Next, the lysate is passed through a membrane that binds the DNA. Contaminants are then washed off the membrane through two washing steps. Finally, the purified DNA is eluted off the membrane in a small volume of an appropriate buffer.

The DNA extract should be free of contaminants, but further purification methods may be used to remove inhibitory substances that remain, if necessary.

This method is appropriate for use on most dried stains, liquid samples, and other biological materials encountered in forensic casework and should be used for samples suspected to contain low levels of DNA as it has been shown to improve recovery of DNA.

## 2. References

- 2.1. Qiagen QIAamp<sup>®</sup> DNA Micro Handbook, Aug. 2003.
- 2.2. I. van Niekerek, C. Snyman, J. van Niekerk, A. Greyling, Efficient purification of genomic DNA from forensic samples using QIAamp<sup>®</sup> Kits, *Qiagen News* 1 (2005) 10-12.
- 2.3. V. Castella, N. Dimo-Simonin, C. Brandt-Casadevall, P. Mangin, Forensic evaluation of the QIAshredder<sup>®</sup>/QIAamp<sup>®</sup> DNA extraction procedure, *Forensic Sci. Int.* 156 (2006) 70-73.
- 2.4. D.J. Johnson, L. Martin, K.A. Roberts, STR-typing of human DNA from human fecal matter using the Qiagen QIAamp<sup>®</sup> Stool Mini Kit, *J. Forensic Sci.* 50 (2005) 802-808.
- 2.5. N. Vandenberg, R.A. van Oorschot, Extraction of human nuclear DNA from feces samples using the QIAamp<sup>®</sup> DNA Stool Mini Kit, *J. Forensic Sci.* 47 (2002) 993-995.
- 2.6. J.M. Butler, Sample collection, DNA extraction, and DNA quantitation, in: *Forensic DNA Typing*, second ed., Elsevier Academic Press, Burlington, 2005, pp 42-50.
- 2.7. P. Gill, A.J. Jeffreys, D.J. Werrett, Forensic application of DNA 'Fingerprints', *Nature* 318 (1985) 577-579.
- 2.8. R. Higuchi, C.H. von Beroldingen, G.F. Sensabaugh, H.A. Erlich, DNA typing from single hairs, *Nature* 332 (1988) 543-546.
- 2.9. C.T. Comey, B.W. Koons, K.W. Presely, J.B. Smerick, C.A. Sobieralski, D.M. Stanley, F.S. Baechtel, DNA extraction strategies for amplified fragment length polymorphism analysis, *J. Forensic Sci.* 39 (1994) 1254-1269.
- 2.10. S.A. Greenspoon, M.A. Scarpetta, M.L. Drayton, S.A. Turek, QIAamp<sup>®</sup> spin columns as a method of DNA isolation for forensic casework, *J. Forensic Sci.* 43 (1998) 1024-1030.

- 2.11. K. Sinclair, V.M. McKechnie, DNA extraction from stamps and envelope flaps using QIAamp<sup>®</sup> and QIAshredder<sup>®</sup>, J. Forensic Sci. 45 (2000) 229-230.
- 2.12. Qiagen, Developmental validation of the QIAamp<sup>®</sup> DNA Investigator Kit, July 2015, available at: <https://www.qiagen.com/us/resources/resourcedetail?id=1d9de4e8-cbd4-4be7-aec2-dd9f79f038fb&lang=en&autoSuggest=true>.

### 3. Equipment

- 3.1. Disposable gloves
- 3.2. Eye protection
- 3.3. Lab coat
- 3.4. Sterile swabs
- 3.5. Sterile water
- 3.6. Disposable scalpels or razor blades
- 3.7. Scissors
- 3.8. Forceps
- 3.9. 70% ethanol or alcohol wipes
- 3.10. 10% bleach solution
- 3.11. QIAamp<sup>®</sup> DNA Micro Kit (Qiagen Catalog # 56304) containing the following:
  - 3.11.1. QIAamp<sup>®</sup> MinElute™ columns
  - 3.11.2. Collection Tubes
  - 3.11.3. Buffer ATL
  - 3.11.4. Buffer AL
  - 3.11.5. Buffer AW1
  - 3.11.6. Buffer AW2
  - 3.11.7. Buffer AE
  - 3.11.8. Carrier RNA
  - 3.11.9. Proteinase K (typically not used)
- 3.12. Invitrogen Proteinase K
- 3.13. Dithiothreitol (DTT)
- 3.14. Pipettes
- 3.15. Disposable aerosol-resistant pipette tips
- 3.16. Microcentrifuge tubes
- 3.17. NAO™ Baskets/QIAGEN<sup>®</sup> Investigator Lyse and Spin and tubes  
**OPTIONAL: DNA IQ™ Spin Baskets**
- 3.18. Benchtop hood
- 3.19. TE<sup>-4</sup> (10mM Tris-HCl , 0.1mM EDTA, pH 8.0)
- 3.20. Bench paper
- 3.21. Thermomixer
- 3.22. Centrifuge
- 3.23. Vortexer

### 4. Safety/Quality Assurance

- 4.1. Disposable gloves shall be worn when handling kit reagents and evidence.

- 4.2. Perform extraction steps, where possible, in a hood to reduce the risk of contamination. Clean surfaces with 10% bleach solution, followed by 70% EtOH, before and after use. After exiting hood, turn on UV light (automatically set for 15 minutes of exposure).
- 4.3. When possible, reference samples will be processed in a designated area after the analysis of evidence samples has been completed.
- 4.4. Use aerosol-resistant pipette tips when transferring liquids containing DNA.
- 4.5. Change pipette tips after transferring any liquids potentially containing DNA.
- 4.6. Record the lot number and expiration date of each reagent used in notes. Do not use the reagents after the expiration date.
- 4.7. Initiate at least one reagent blank as the final sample of the set of extractions.
- 4.8. Lab coat and eye protection must be worn at all times while performing this procedure.
- 4.9. Only tubes associated with one DNA extract shall be open at a time.
- 4.10. Exercise caution when opening tubes.
- 4.11. The laboratory bench surface shall be cleaned before use with 10% bleach solution or other sanitizing agent and may be followed by 70% ethanol. Fresh bench paper shall then be placed on the surface prior to evidence examination.
- 4.12. Final DNA extract tubes shall be labeled, at a minimum, with case number, item number, and analyst's initials.
- 4.13. Samples thought to contain lower levels of DNA will be handled before those thought to contain large amounts of DNA.
- 4.14. Minor deviations from the protocol may be made at the analyst's discretion based on the analyst's training and experience and shall be indicated in the analyst's notes. Significant deviations from the protocol must be approved by the technical leader.

## **1. Sample Preparation**

- 1.1. Any utensils used to cut or manipulate the swabs or other types of evidence must be cleaned between uses with 10% bleach solution and followed by 70% ethanol or alcohol wipe. Alternatively, single-use disposable razor blades may be used.
- 1.2. To maximize the recovery of the lysate, cuttings of swab tips or evidence substrate should be placed in a clean NAO™ Basket/QIAGEN® Investigator Lyse and Spin basket with tube. Alternatively, a clean microcentrifuge tube may be used.
  - 1.2.1. Cuttings from biological stains/substrate (clothing, carpet, etc.) should be approximately 5 mm x 5 mm in size. Some specimens may require cuttings of differing sizes depending on the type, condition and concentration of the biological material. Larger sized cuttings may be cut into smaller pieces before placing them into the tube.
- 1.3. For liquid samples, place a portion of the liquid (1-100 µL) directly into a clean microcentrifuge tube.
- 1.4. Hair Roots
  - 1.4.1. Prior to processing any hairs for DNA, consult with a trace evidence examiner.
  - 1.4.2. When appropriate, gently rinse hair with 70% ethanol followed by sterile water prior to cutting.
  - 1.4.3. Cut a 0.5-1cm piece of the root end of the hair and put it in a microcentrifuge tube containing the extraction reagents.
  - 1.4.4. If hair is not cleaned with ethanol and water, generate a control portion of hair by cutting another 0.5-1 cm piece of hair (non-root) and process it alongside the sample.
- 1.5. For reference samples, cut a small portion of blood card (up to 5 mm<sup>2</sup>), swab tip, or other reference material and place in a clean microcentrifuge tube (or in a clean NAO™ Basket/QIAGEN® Investigator Lyse and Spin basket with tube if recovery of lysate is a

priority).

## 6. Procedure

- 6.1. Check the reagent logs or the reagent bottles to ensure that Buffer AW1, Buffer AW2, and carrier RNA have been appropriately prepared. Prior to use, Buffers ATL, AW1, and AW2 must contain no precipitates. If precipitates have formed, gently heat the bottles prior to dispensing the reagents.
- 6.2. A Reagent Blank shall be initiated as the last sample in the set of samples. The Reagent Blank shall contain all the liquid reagents contained in the evidentiary samples except for the biological material. The Reagent Blank shall be handled in a manner that is identical to the evidentiary samples and use the most sensitive volumes and steps used with the evidentiary samples. For example, if carrier RNA is used with only a few of the evidentiary samples being extracted in a set, carrier RNA will be added to the Reagent Blank as well.
  - 6.2.1. If the analyst determines that two or more evidentiary extracts may be combined during the concentration step, the same number of Reagent Blanks shall be initiated at the DNA extraction step. For example, if the analyst determines that the extracts from three sets of swabs from a firearm may be combined and concentrated later in the analysis, then the analyst shall initiate three Reagent Blanks at the DNA extraction step.
  - 6.2.2. If an NAO™ Basket/QIAGEN® Investigator Lyse and Spin basket is used, the tips of two swabs should be placed in the Reagent Blank to facilitate the recovery of the entire volume of sample.

### Cell Lysis

- 6.3. For **non-hair samples**, add **300-400 µL of Buffer ATL** and **20 µL of ProK** (20 mg/mL) to the sample tube.
  - 1.5.1. The volume of ATL buffer may be increased to ensure that the sample is completely immersed in the lysis solution.  
**NOTE: A 20:1 ratio of ATL:Pro K must be maintained. For example: 500 µL ATL : 25 µL Pro K.**
- 6.4. For **hair samples**, add **300 µL of Buffer ATL**, **20 µL of ProK** (20 mg/ml), and **20 µL of 1M DTT** to the sample tube.
- 6.5. Vortex for approximately 10 seconds.
- 6.6. Place samples in a thermomixer and incubate at 56° C with shaking at 900 rpm for at least 3 hours.  
**NOTE: Samples may be incubated overnight; however, incubation times greater than 18 hours have been shown to decrease DNA yield.**
- 6.7. If NAO™ Basket/QIAGEN® Investigator Lyse and Spin basket is used for improved lysate recovery, centrifuge samples for up to 3 minutes at 18,000 x g. Baskets and substrate may be discarded at this point. Continue at step 6.9.
- 6.8. Briefly centrifuge the samples to remove condensation from inside the lid.
- 6.9. Add a volume of **Buffer AL** equal to **twice** the volume of Buffer ATL used. For example, if 400 µl of Buffer ATL was used in step 6.3, then add 800 µl of Buffer AL.

- 6.10. **1  $\mu\text{L}$  of carrier RNA** (1  $\mu\text{g}/\mu\text{L}$ ) can be added at this point if, due to the concentration or condition of the biological material, it is determined by the analyst that carrier RNA may aid in the recovery of the DNA.
- 6.11. Vortex for approximately 10 seconds.
- 6.12. Place the tubes in a thermomixer and incubate at 70° C with shaking at 900 rpm for at least 10 minutes.  
**NOTE: Any white precipitate that formed when the Buffer AL was added should disappear during this incubation step.**
- 6.13. Briefly centrifuge the samples to remove condensation from the inside of the lid.
- 6.14. If still present, the solid substrate can be removed and discarded.  
**OPTIONAL: If maximum recovery of lysate is a priority, and NAO™ Baskets/QIAGEN® Investigator Lyse and Spin baskets are unavailable, the solid substrate can be placed in a DNA IQ™ Spin Basket for centrifugation.**
  - 6.14.1. If DNA IQ™ Spin Baskets were used, centrifuge tubes for 2 minutes at full speed.
  - 6.14.2. Discard basket and substrate after centrifugation.

#### Bind DNA

- 6.15. Carefully transfer up to 700  $\mu\text{L}$  of the supernatant from the microcentrifuge tube to a QIAamp MinElute™ column without wetting the rim of the column.
- 6.16. Centrifuge the column at 6000 x g for 1 minute. Place the MinElute™ column in a clean 2 mL collection tube and discard the collection tube containing the flow-through.
- 6.17. Transfer any remaining supernatant from the microcentrifuge tube to the QIAamp MinElute™ column without wetting the rim of the column.
- 6.18. Centrifuge the column at 6000 x g for 1 minute. Place the MinElute™ column in a clean 2 mL collection tube and discard the collection tube containing the flow-through.

#### Wash DNA

- 6.19. Carefully open the QIAamp MinElute™ column and add **500  $\mu\text{L}$  of Buffer AW1** without wetting the rim. Close the lid and centrifuge at 6000 x g for 1 minute.
- 6.20. Place the QIAamp MinElute™ column in a clean 2 mL collection tube and discard the collection tube containing the flow-through.
- 6.21. Carefully open the QIAamp MinElute™ column and add **700  $\mu\text{L}$  of Buffer AW2** without wetting the rim. Close the lid and centrifuge at 6000 x g for 1 minute.
- 6.22. Place the QIAamp MinElute™ column in a clean 2 mL collection tube and discard the collection tube containing the flow-through.
- 6.23. Centrifuge at full speed for 3 minutes to air-dry the membrane completely.
- 6.24. Place the QIAamp MinElute™ column in a clean microcentrifuge tube and discard the collection tube containing the flow-through.

#### Elute Purified DNA

- 6.25. Carefully apply **20-50  $\mu\text{L}$  of TE<sup>-4</sup>** to the center of the membrane.
- 6.26. Incubate at room temperature for 1-5 minutes.
- 6.27. Centrifuge at full speed for 1 minute.

**RECOMMENDED: Repeat steps 6.25 through 6.27 to increase yield.**

- 6.28. Remove the QIAamp MinElute™ column and cap the tube. The QIAamp MinElute™ column can be discarded. The DNA extract may be concentrated at this point or may proceed to quantitation directly.