# Forensic Biology Section

Extraction of DNA with QIAamp Micro Kit

## 1. Scope

1.1. This procedure outlines the steps for isolating DNA from nucleated cells using the QIAamp DNA Micro kit, consisting of a column with a silica-gel membrane placed inside a microcentrifuge tube.

1.2. There are 6 steps:

- First, the samples are lysed under highly denaturing conditions at elevated temperatures in the presence of Proteinase K and ATL Buffer.
- Second, to allow optimal binding of the DNA to the silica-gel membrane, AL Buffer and ethanol are added to the lysate to create a low pH and high salt concentration.
- Third, the extracts are transferred into the QIAamp MinElute column and centrifuged, allowing any DNA present to bind to the silica-gel membrane while proteins and other contaminants pass though the column.
- Fourth and fifth, residual contaminants are washed away with AW1 and AW2 Buffer.
- Sixth, the DNA is eluted from the membrane with $TE^{-4}$.

1.3. AE Buffer from the QIAamp kit is NOT recommended because it may cause inhibition when amplifying 10µl or more of DNA extract; extracts with AE Buffer can be concentrated and brought up to volume with $TE^{-4}$ to compensate for the extra EDTA in the AE Buffer.

1.4. If the presence of inhibitors is suspected in a DNA extract, see "Cleanup of DNA Extracts" in this procedure to potentially improve the purity of an extract.

## 2. Safety

2.1. **CHEMICAL HAZARD**: **Do NOT combine bleach with any extraction waste or the AL and AW1 buffers**; these contain guanidine hydrochloride (or guanidinium chloride) which can react violently with oxidizing agents or strong alkalis and produce toxic chlorine gas.

2.2. **Use alcohol (not bleach) to clean up spills of QIAamp reagents and extraction waste.**

2.3. Do not discard liquid waste down sink drains. Transfer QIAamp reagents and extraction waste to collection bottle in Extraction Lab for chemical waste disposal.

## 3. Specimens

The QIAamp kit is suitable for biological material on swabs, filter, or FTA, including:

- Blood
- Semen
- Saliva
- Muscle
- Skin
- Hair root
- Epi swabs" or "Touch DNA"
- Urine
- Bone

3.1. Evidentiary samples may be limited. Add the maximum amount of evidentiary sample that can fit in a tube if it is thought very little biological material is present, trying to retain sufficient sample for replicate analysis if practical. Epi/Touch DNA swabs are usually consumed.

## 4. Reagents

- QIAamp DNA Micro Kit (MinElute columns, buffers, and proteinase K)
- Tris-EDTA ($TE^{-4}$) = 10mM Tris-HCl, 0.1mM EDTA, pH 8.0
- Dithiothreitol (DTT) = 1 Molar
- Absolute Ethyl Alcohol (200 proof)
- Molecular biology grade Water
- Sperm Wash Buffer

## 5. Instrumentation

- Thermomixer (or dry block incubators) set to 56 °C, 70 °C, and 37 °C
- Centrifuge or Microcentrifuge
- Vortex

## 6. Procedural Notes

6.1. Equilibrate samples and columns to room temperature (~15-25 °C) before use.

6.2. Gently mix the AW1 and AW2 Buffers before use. If precipitates are visible in any buffers (typically in AL or ATL), warm the bottle and gently mix until precipitates go back into solution.

6.3. When adding buffers to the QIAamp columns, avoid getting liquid on the column's rim.

6.4. At the elution step, add the Tris-EDTA ($TE^{-4}$) to the membrane, not the gasket at the periphery, to ensure the bound DNA is eluted from the column.

6.5. Eluted DNA extracts can be stored in a refrigerator (1 °C – 8 °C) for short-term storage, stored in a freezer (-10 °C or colder) for long-term storage, or processed in downstream applications immediately.

6.6. This protocol is written for the use of 1.5ml microcentrifuge tubes. If using larger tubes (e.g. 15ml conical tubes), scale up the proportion of each reagent as needed.

## 7. Extracting Samples Other than Sperm, Tissue, Liquid Blood or Urine

7.1. Lyse samples according to the following steps:

7.1.1. Cigarette butts: Cut a ring of paper from the filter end (~0.5 to 1cm) and place in a microcentrifuge tube. Add 300µl of ATL Buffer and 20µl of Proteinase K and mix by vortexing for 10 seconds. *Continue with step 7.2.*

7.1.2. Envelopes or stamps: Cut a 0.5 to 2.5$cm^2$ sample from the envelope or stamp. Transfer to a microcentrifuge tube. Add 300µl of ATL Buffer and 20µl of Proteinase K and mix by vortexing for 10 seconds. *Continue with step 7.2.*

7.1.3. Hair roots: Add 300µl of ATL Buffer, 20µl of Proteinase K, and 20µl of 1M DTT to a microcentrifuge tube. Cut off a 0.5-1cm piece of hair including the hair root and transfer it to the microcentrifuge tube. Mix by vortexing for 10 seconds. *Continue with step 7.2.*

7.1.4. Fingernail clippings: Transfer clippings to a microcentrifuge tube. Add 300µl of ATL Buffer, 20µl of Proteinase K, and mix by vortexing for 10 seconds. *Continue with step 7.2.*

7.1.5. Epi swabs: If possible, separate the swab head from the shaft and place in a 1.5ml or 2ml microcentrifuge tube. Add 400µl of ATL Buffer and 20µl of Proteinase K and mix by vortexing for 10 seconds. *Continue with step 7.2.*

**Optional: To verify the presence of human hemoglobin using HemaTrace test,** incubate the substrate in up to 1ml of $TE^{-4}$ to elute the stain (15 minutes or more), centrifuge for 5 minutes at full speed to pellet any cells, test approximately 80µl of the supernatant (see "ABAcard HemaTrace Human Blood Detection" method), discard the remainder of the supernatant without disturbing the cell pellet, and *continue with step 7.1.6 below.*

7.1.6. Blood or saliva stains: Cut out stained material and place in a microcentrifuge tube (**see step above for HemaTrace directions**). Add 300µl of ATL Buffer and 20µl of Proteinase K and mix by vortexing for 10 seconds. *Continue with step 7.2.*

7.1.7. FTA or filter paper: Using a clean punch, remove a sample from the middle of the stain and place it in a microcentrifuge tube. Add 300µl of ATL Buffer and 20µl of Proteinase K and mix by vortexing for 10 seconds. Note**:** make several punches in clean paper between samples to prevent transfer of biological material from one sample to the next. *Continue with step 7.2.*

7.1.8. Sperm cell pellets with very low # sperm: (when the # of sperm cells is too low to do effective differential, e.g., less than 10 sperm per slide in sperm search). Add 300µl of ATL Buffer, 20µl of Proteinase K, and 20µl of 1M DTT to microcentrifuge tube containing cell pellet. Mix by vortexing for 10 seconds. *Continue with step 7.2*

7.2. Place the microcentrifuge tube in a thermomixer and incubate at 56 ºC for at least 1 hour (shaking or vortexing to mix).

7.3. Briefly centrifuge to remove any condensation from the inside of the lids.

Note: The thermomixer should be set to 70ºC at this time.

7.4. Add 300µl (or 400µl for swabs) of AL Buffer to each tube and mix by vortexing for 10 seconds.

7.5. OPTION: If tubes foam, spin tubes before putting into Thermomixer and do not vortex again.

7.6. Place the tubes in the thermomixer and incubate at 70 ºC for 10 minutes (with shaking/vortexing).

7.7. Briefly centrifuge to remove any condensation from the inside of the lids.

7.8. Add 150µl (or 200µl for swabs) of absolute ethanol to each tube and mix by vortexing for 15 seconds.

7.9. Centrifuge at full speed for 1 minute.

7.10. Transfer the **first** 500µl of the supernatant into labeled QIAamp columns and centrifuge at 8000 rpm for one minute.

7.11. Transfer the **remaining** supernatant into the corresponding QIAamp columns and centrifuge again at 8000 rpm for one minute.

Note: If the liquid has not completely passed through the membrane after centrifuging, centrifuge the QIAamp column again at a higher speed until the column is empty. Also, some liquid may remain in substrates such as swabs and cloth; recover this liquid by placing the substrate in a Spin Basket and centrifuging at full speed for 2 minutes. Add any recovered liquid to the column and centrifuge at 8000 rpm again.

7.12. Move the columns to clean collection tubes (discard the previous collection tubes).

7.13. Add 500µl of AW1 Buffer to each QIAamp column and centrifuge at 8000 rpm for one minute.

7.14. Move the columns to clean collection tubes (discard the previous collection tubes).

7.15. Add 500µl of AW2 Buffer to each QIAamp column and centrifuge at 8000 rpm for one minute.

7.16. Move the columns to clean collection tubes (discard the previous collection tubes).

7.17. Centrifuge at full speed for three minutes to dry the membrane completely.

7.18. Move the columns to appropriately labeled microcentrifuge tubes (discard the previous collection tubes). Add 20-200µl of $TE^{-4}$ to the center of each membrane depending on the desired final volume of extract.

7.19. Incubate at room temperature for 5 minutes, then centrifuge at full speed for 1 minute. DNA present on the membrane should elute into the microcentrifuge tube.

7.20. The extract can now be stored or processed in downstream applications.

## 8. Differential Extraction of Samples Containing Sperm Cells

8.1. Starting from cutting or swab:

8.1.1. Dissect swab or fabric and place in a labeled Spin Tube (2.0ml).

8.1.2. Add 800µl of TE buffer to each tube . Vortex for at least 10 seconds (and/or agitate with a sterile pipette tip) to aid in releasing cells from the substrate.

8.1.3. Incubate for 30-60 minutes at room temperature, or longer in a refrigerator.

8.1.4. After incubating, vortex the tube for at least 10 seconds (and/or agitate with a sterile pipette tip).

8.1.5. Transfer the substrate with a sterile pipette tip or forceps into a Spin Basket and place back in the top of the corresponding Spin Tube.

8.1.6. Centrifuge at full speed for 5 minutes to elute the liquid and cells from the substrate in the basket.

8.1.7. Remove and discard the Spin Basket and substrate (unless there is no other substrate to go back to – in those cases the substrate should be preserved).

**Optional: To verify the presence of human hemoglobin using HemaTrace test,** test approximately 80µl of the supernatant (see "ABAcard HemaTrace Human Blood Detection" method) without disturbing the cell pellet, and *continue with step 8.1.8 below.*

8.1.8. Without disturbing the pellet, remove all but approximately 50µl of supernatant (or 2x volume of pellet, whichever is greater).

8.1.9. Suspend the cell debris pellet thoroughly in the remaining supernatant by pipetting up and down vigorously.

Optional: Remove an aliquot equal to one-tenth of the volume in the tube to verify and quantify the cell pellet content (see the "Microscopic Examination of Cell Debris" method).

8.2. Starting from Cell Pellet

8.2.1. Add 100µl of molecular biology grade water to the approximately 50µl of cell debris pellet (final volume should be approximately 150µl).

8.2.2. Add 6µl of Proteinase K to each tube and gently vortex.

8.2.3. Incubate at 37 °C for approximately 1 hour to lyse any epithelial cells, but for no more than 2 hours to minimize the lysis of sperm cells.

8.2.4. Centrifuge at full speed for 5 minutes. This original tube is now labeled with an "S", "Sp", or "Sperm" (will contain the resulting sperm pellet).

8.2.5. Without disturbing the sperm pellet, transfer all but approximately 50µl of the supernatant to a new tube, labeled with an "E", "Ep", or "Epi" (containing any lysed epithelial cell DNA).

8.2.6. Wash the sperm-pellet ("S" tube) with Sperm Wash Buffer as follows:

8.2.6.1. Suspend the sperm pellet in 500µl Sperm Wash Buffer and vortex.

8.2.6.2. Centrifuge at full speed for 5 minutes.

8.2.6.3. Without disturbing the pellet, remove and discard as much of the supernatant as possible.

8.2.7. Repeat Step 8.2.6 an additional 2-3 times, for a maximum of 4 washes.

8.2.8. Wash the sperm-pellet ("S" tube) once with molecular biology grade water as follows:

8.2.8.1. Suspend the pellet in 500µl to 1 ml molecular biology grade water and vortex. Centrifuge at full speed for 5 minutes.

8.2.8.2. Without disturbing the pellet, remove and discard as much of the supernatant as possible. *Continue with step 8.3.*

Optional: Suspend the pellet and remove one-tenth aliquot for a microscopic examination to assess the quantity of sperm and the efficiency of removing epithelial cells (see the "Microscopic Examination of Cell Debris Pellets" method).

8.3. QIAamp Column Purification

8.3.1. Add 300µl of ATL Buffer to the tubes containing the "S" and "E" fractions.

8.3.2. Add 20µl of DTT and 20µl of Proteinase K to the "S" fraction (do not add DTT or Proteinase K to the "E" fraction).

8.3.3. Place both tubes in a thermomixer and incubate at 56 ºC for at least 1 hour (shaking or vortexing to mix).

8.3.4. Briefly centrifuge to remove any condensation from the inside of the lids.

Note: The thermomixer should be set to 70ºC at this time.

8.3.5. Add 300µl of AL Buffer to each tube and mix by vortexing for 10 seconds.

8.3.6. OPTION: If tubes foam, spin tubes before putting into Thermomixer and do not vortex again.

8.3.7. Place the tubes in a thermomixer and incubate at 70 ºC for 10 minutes (with shaking/vortexing).

8.3.8. Briefly centrifuge to remove any condensation from the inside of the lids.

8.3.9. Add 150µl of absolute ethanol to each tube and mix by vortexing for 15 seconds.

8.3.10. Centrifuge at full speed for 1 minute.

8.3.11. Transfer the **first 500µl** of the supernatant into labeled QIAamp columns and centrifuge at 8000 rpm for one minute.

8.3.12. Transfer the **remaining** supernatant into the corresponding columns and centrifuge again at 8000 rpm for one minute (discard the tubes containing the substrate).

Note: If the liquid has not completely passed through the membrane after centrifuging, centrifuge the QIAamp column again at a higher speed until the column is empty.

8.3.13. Move the columns into clean collection tubes (discard the previous collection tubes)

8.3.14. Add 500µl of AW1 Buffer to each column and centrifuge at 8000 rpm for one minute.

8.3.15. Move the columns to clean collection tubes (discard the previous collection tubes).

8.3.16. Add 500µl of AW2 Buffer to each column and centrifuge the columns at 8000 rpm for one minute.

8.3.17. Move the columns to clean collection tubes (discard the previous collection tubes).

8.3.18. Centrifuge at full speed for three minutes to dry the membrane completely.

8.3.19. Move the columns to appropriately labeled microcentrifuge tubes (discard the previous collection tubes). Add 20-200µl of $TE^{-4}$ to the center of the membrane.

8.3.20. Incubate at room temperature for 5 minutes, then centrifuge at full speed for 1 minute. DNA present on the membrane should elute into the microcentrifuge tube.

8.3.21. The extract can now be stored or processed in downstream applications.

## 9. Extracting Samples of Liquid Blood

9.1. Pipet 1-50µl of well-mixed whole blood into a microcentrifuge tube.

9.2. Add ATL Buffer to each tube to a final volume of 100µl.

9.3. Add 10µl of Proteinase K to each tube.

9.4. Add 100µl of AL Buffer to each tube and mix by vortexing for 15 seconds.

9.5. Place the tubes in the thermomixer and incubate at 56 ºC for 10 minutes (with mixing/vortexing).

9.6. Briefly centrifuge to remove any condensation from the inside of the lids.

9.7. Add 50µl of absolute ethanol to each tube and mix by vortexing for 15 seconds.

9.8. Incubate for 3 minutes at room temperature.

9.9. Briefly centrifuge to remove any liquid from inside the lids.

9.10. Transfer the entire sample to a labeled QIAamp column and centrifuge at 8000 rpm for 1 minute.

9.11. Move the columns to clean collection tubes (discard the previous collection tube).

9.12. Add 500µl of AW1 Buffer to each column and centrifuge at 8000 rpm for 1 minute.

9.13. Move the columns to clean collection tubes (discard the previous collection tube).

9.14. Add 500µl of AW2 Buffer to each column, and centrifuge at 8000 rpm for 1 minute.

9.15. Move the columns to clean collection tubes (discard the previous collection tube).

9.16. Centrifuge at full speed for three minutes to dry the membrane completely.

9.17. Move the columns to appropriately labeled microcentrifuge tubes (discard the previous collection tubes). Add 20-200µl of $TE^{-4}$ to the center of each membrane depending on the desired final volume of extract.

9.18. Incubate at room temperature for 5 minutes, then centrifuge at full speed for 1 minute. DNA present on the membrane should elute into the microcentrifuge tube.

9.19. The extract can now be stored or processed in downstream applications.

Note: If the color of the extracted sample is a red brown color, the potential for inhibition (from heme) should be considered.

## 10. Extracting Tissue Samples

10.1. Place a very small tissue sample in a microcentrifuge tube.

10.2. Add 180µl of ATL Buffer to each tube.

10.3. Add 20µl of Proteinase K to each tube and mix by vortexing for 15 seconds.

10.4. Place the tubes in a thermomixer and incubate at 56 °C for 4-6 hours or overnight (with shaking/vortexing).

10.5. Briefly centrifuge to remove any condensation from the inside of the lids.

Note: The thermomixer should be set to 70ºC at this time.

10.6. Add 200µl of AL Buffer to each tube and mix by vortexing for 15 seconds.

10.7. OPTION: If tubes foam, spin tubes before putting into Thermomixer and do not vortex again.

10.8. Place the tubes in a thermomixer and incubate at 70 ºC for 10 minutes (with shaking/vortexing).

10.9. Briefly centrifuge to remove any condensation from the inside of the lids.

10.10. Add 200µl of absolute ethanol to each tube and mix by vortexing for 15 seconds.

10.11. Incubate for 5 minutes at room temperature (~15-25 °C).

10.12. Briefly centrifuge to remove any condensation from the inside of the lids.

10.13. Transfer the **first** 500µl of the supernatant into labeled QIAamp columns and centrifuge at 8000 rpm for one minute (discard the tubes containing any substrate).

10.14. Transfer the **remaining** supernatant into the corresponding columns and centrifuge again at 8000 rpm for one minute.

Note: If the liquid has not completely passed through the membrane after centrifugation, centrifuge again at a higher speed until the column is empty.

10.15. Move the columns to clean collection tubes (discard the previous collection tubes).

10.16. Add 500µl of AW1 Buffer to each column and centrifuge at 8000 rpm for one minute.

10.17. Move the columns to clean collection tubes (discard the previous collection tubes).

10.18. Add 500µl of AW2 Buffer to each column and centrifuge at 8000 rpm for one minute.

10.19. Move the columns to clean collection tubes (discard the previous collection tube).

10.20. Centrifuge at full speed for three minutes to dry the membrane completely.

10.21. Move the columns to appropriately labeled microcentrifuge tubes (discard the previous collection tube). Add 20-200µl of $TE^{-4}$ to the center of each membrane depending on the desired final volume of extract.

10.22. Incubate at room temperature for 5 minutes, then centrifuge at full speed for 1 minute. DNA present on the membrane should elute into the microcentrifuge tube.

10.23. The extract can now be stored or processed in downstream applications.

## 11. Extracting Liquid Urine Samples

11.1. Transfer up to 1ml of urine to a microcentrifuge tube and centrifuge at full speed for 2 minutes. If starting with a larger volume of urine, pellet cells in a 15ml or 50ml conical tube by centrifuging at full speed for at least 5 minutes.

11.2. Without disturbing the pellet, discard the supernatant and add 500µl $TE^{-4}$ and suspend the pellet by mixing and vortexing.

11.3. Centrifuge at full speed for 2 minutes.

11.4. Discard the supernatant, add 300µl ATL Buffer and 20µl of Proteinase K to the pellet, and mix by vortexing for 10 seconds. Note: Adding 20 µl DTT may increase yield, since urine can contain sperm cells.

11.5. Place the tubes in a thermomixer and incubate at 56 °C for 1 hour (with mixing/vortexing).

11.6. Briefly centrifuge to remove any condensation from the inside of the lids.

11.7. Add 300µl AL Buffer and 50µl of absolute ethanol to each tube and mix by vortexing for 10 seconds.

11.8. Briefly centrifuge to remove any liquid from the inside of the lids.

11.9. Transfer the **first** 500µl of the extract to labeled QIAamp columns and centrifuge at 8000 rpm for one minute (discard the tubes containing any substrate).

11.10. Transfer the **remaining** supernatant into the corresponding columns and centrifuge again at 8000 rpm for one minute

11.11. Move the columns to clean collection tubes (discard the previous collection tubes).

Note: If the liquid has not completely passed through the membrane after centrifuging, centrifuge again at a higher speed until the column is empty.

11.12. Add 500µl of AW1 Buffer to each column and centrifuge at 8000 rpm for one minute.

11.13. Move the columns to clean collection tubes (discard the previous collection tubes).

11.14. Add 500µl of AW2 Buffer to each column and centrifuge at 8000 rpm for one minute.

11.15. Move the columns to clean collection tubes (discard the previous collection tubes).

11.16. Centrifuge at full speed for three minutes to dry the membrane completely.

11.17. Place the columns into appropriately labeled microcentrifuge tubes (discard the previous collection tubes). Add 20-200µl of $TE^{-4}$ to the center of each membrane depending on the desired final volume of extract.

11.18. Incubate at room temperature for 5 minutes, then centrifuge at full speed for 1 minute. DNA present on the membrane should elute into the microcentrifuge tube.

11.19. The extract can now be stored or processed in downstream applications.

## 12. Cleanup of DNA Extracts (to remove potential inhibitors)

12.1. Transfer up to 100µl of DNA extract to a microcentrifuge tube. If less DNA extract is used, add molecular biology grade water for a final volume of 100µl in each tube.

12.2. Add 10µl of AW1 Buffer to each tube and 250µl of AW2 Buffer to the tube and mix by vortexing for 15 seconds.

12.3. Transfer to a labeled QIAamp column and centrifuge at 8000 rpm for one minute.

12.4. Move the column to a clean collection tube (discard the previous collection tube).

12.5. Add 500µl of AW2 Buffer to the column and centrifuge at 8000 rpm for one minute.

12.6. Move the column to a clean collection tube (discard the previous collection tube).

12.7. Centrifuge at full speed for three minutes to dry the membrane completely.

12.8. Move the column to an appropriately labeled microcentrifuge tube (discard the previous collection tube). Add 20-200µl of $TE^{-4}$ to the center of the membrane depending on the desired final volume of extract.

12.9. Incubate at room temperature for 5 minutes, then centrifuge at full speed for 1 minute. DNA present on the membrane should elute into the microcentrifuge tube.

12.10. The extract can now be stored or processed in downstream applications.