DNA EXTRACTION OF DRIED STAINS USING THE QIAAMP DNA BLOOD KIT - 96 WELL FORMAT

Background

This protocol allows for the isolation of DNA from blood which has been spotted and dried on FTA / filter paper. With this procedure, up to 96 samples can be extracted at one time.

Summary of Procedure

In a 96 well format, one to three 3mm punches per sample are subjected to Protease and Phosphate Buffered Saline (PBS). The cell lysate is then loaded into the QIAamp spin column (96 well format). DNA is absorbed onto the silica membrane while protein and other contaminants, which may inhibit PCR and other enzymatic reactions, are not retained on the membrane under these conditions. The DNA binding membrane is washed to completely remove any residual contaminants without any effect on DNA binding. Purified DNA is eluted from the QIAamp spin column in AE buffer.

Sample Handling

All biological samples and DNA must be treated as potentially infectious. Appropriate sample handling and disposal techniques should be followed. See:

- ***Safety Manual***, *Universal Precautions*
- ***Quality Assurance Manual***, *General Sample Control and Forensic Sample Preservation Policy*
- ***Analytical Procedures Manual***, **Forensic Evidence Handling**

Warnings and Precautions

Buffer AL and Buffer AW1 contain chaotropic salt, which is an irritant. Take appropriate laboratory safety measures and wear gloves when handling. These reagents are not compatible with disinfecting agents which contain bleach.

Buffer AW2 contains sodium azide as a preservative. Sodium azide is highly toxic and may react explosively with lead and copper drain pipes. Take appropriate safety measures and wear gloves when handling. Dispose of azide containing solutions according to laboratory waste-disposal guidelines.

Reagents and Materials

See **Appendix B** for reagent preparation

QIAGEN QIAamp 96 DNA Blood Kit
- QIAamp 96 Plates
- S-Blocks
- Round-Well Blocks
- AirPore Tape
- Caps for Round-Well Blocks and Collection Tubes
- Racks of Elution Microtubes
- QIAGEN Protease
- Protease solvent
- Buffer AL
- Buffer AW1*
- Buffer AW2*
- Buffer AE

96-Well Plate Register

*These reagents are concentrates and must have the appropriate ethanol volume added before beginning the procedure.

Biosafety Cabinet
Bench Paper
Centrifuge 4-15C
Vortex
Oven
3mm (1/8") Hole Punch
Forceps
Disposable Pipettes, 10mL and 25mL
Multichannel Pipette (40uL and 300uL)
Single Channel Pipette (40uL and 200uL)
Universal Tips, sterile
Filtered Tips, sterile
300uL Pipette Tips, sterile
Reagent Reservoirs
Gloves
Lab Coat
KimWipes
Ethanol (96-100%)
MBG Water
Phosphate Buffered Saline (PBS) 1X
Conflikt
0.4M HCl

Reagents and Materials – Storage and Handling

All reagents and materials are kept under sterile conditions. Store all reagents according to the manufacturers' recommendations.

Do not use reagents beyond the listed expiration dates. Date and initial all reagents when put in use. Record in the **Reagent Log**.

Quality Control

Use of the **Qiagen Dried Stain 96-Well Extraction Worksheet** is required for documentation. All information must be completed.

Positive Control

Extraction Positive Control (Optional): Used to ensure that the extraction, amplification and typing procedures are working as expected.

Negative Control

Extraction Negative Control: Reagent negative control(s) are processed and run with each set of extractions. This negative control consists of all reagents used in the procedure but contains no DNA sample.

Procedure

Before Starting:

- When using the 96 well microplate centrifuge, be sure to use a corresponding balance.
- Line the Biosafety Cabinet with bench paper.
- Ensure that Buffer AW1 and AW2 have had the appropriate volumes of ethanol added to the solution.
- If a precipitate has formed in the Buffer AL, dissolve it by incubating at 70°C.

- Pre-heat oven to 70°C.
- Be sure to place a weight on top of the round-well block to prevent the lids from popping off during incubation in the oven.

1. Using a 3mm hole punch, punch one to three 3mm punches for each sample onto a kimwipe. Make sure the punches are taken from the center of the stain. Using forceps, fold the punches and place them into the appropriate well of the round well block. Label plate for later identification.

Note: If processing a partial plate, seal unused wells of the round well block with tape.

2. Add 195uL of PBS to each well, cap the wells with 8-strip caps provided, mix and briefly centrifuge to ensure all samples are submerged in liquid, and incubate at 70° C for one hour.

Note: Be sure to place a weight on top of the round well block to prevent the caps from popping off.

Note: Keep the oven at 70° C after incubation. It will be needed again later.

3. Centrifuge briefly to collect any solution from the caps. Allow the centrifuge speed to reach at least 3000 rpm and then stop the centrifuge.

4. Add 20uL QIAGEN Protease to each sample, cap and mix thoroughly. Briefly spin to remove liquid from caps. Allow the centrifuge speed to reach at least 3000 rpm and then stop the centrifuge.

5. Add 200uL Buffer AL to samples. Seal the wells using 8-strip caps provided. Mix thoroughly by shaking vigorously **with both hands** for 15 seconds.

Note: Shaking the block with only one hand will result in less efficient mixing in the wells on the side of the block held in the hand, with lower yields being obtained from those wells. Simply inverting the round well block several times will not be sufficient to initiate efficient lysis. Similarly, vortexing, or placing the plate on a shaker will not ensure complete cell lysis.

6. Centrifuge briefly to collect any solution from the caps. Allow centrifuge speed to reach at least 3000 rpm, and then stop the centrifuge.

7. Incubate at 70° C for at least 10 minutes. Place a weight on top of the round well blocks to prevent lids from popping off. Longer incubation times have no effect on yield or quality. After this step, the oven may be turned off.

8. Centrifuge briefly to collect any solution from the caps. Allow centrifuge speed to reach at least 3000 rpm, and then stop the centrifuge.

9. Remove the caps and add 200uL of ethanol (96-100%) to each well.

10. Seal the wells using new caps for the round well blocks. Shake **vigorously** for 15 seconds.

11. Centrifuge briefly to collect any solution from the caps. Allow the centrifuge speed to reach at least 3000 rpm, and then stop the centrifuge.

12. Place QIAamp 96 plate on top of an S-block. Label the plate for later identification.

Note: Be certain that the plate orientation is the same.

13. Carefully apply the mixture (620uL per well) from the round well block to the corresponding well in the QIAamp 96 plate. Do not transfer the punch to the QIAamp 96 plate. Take care not to wet the rims of the plate; this could result in cross contamination. Discard the round well block in the Biohazard trash.

14. Seal the QIAamp 96 plate with an airpore tape sheet. Load the S-block and QIAamp 96 plate onto the centrifuge carrier, and then place it in the rotor bucket. Centrifuge at 6000 rpm for 4 minutes.

Note: If extracting one tray, a QIAamp 96 plate needs to be prepared with MGB Water mimicking the sample plate to be used as a balance in the centrifuge. Place the sample plate in the rotor bucket with position A1 in the top left corner. Rotate the rotor 180° and place the balance plate in the rotor bucket with position A1 in the top left corner. The plates will be mirror images of one another.

15. Remove the tape. Add 500uL of Buffer AW1 to each well.

16. Seal the QIAamp 96 plate with an airpore tape sheet. Load the S-block and QIAamp 96 plate onto the centrifuge carrier, and then place it in the rotor bucket. Centrifuge at 6000 rpm for 2 minutes.

17. Remove the tape. Add 500uL of Buffer AW2 to each well.

18. Seal the QIAamp 96 plate with an airpore tape sheet. Load the S-block and QIAamp 96 plate onto the centrifuge carrier, and then place it in the rotor bucket. Centrifuge at 6000 rpm for 15 minutes.

Note: The heat generated during centrifugation allows for evaporation of any residual ethanol on the membrane.

19. Empty the waste and repeat steps 17 and 18 for a second AW2 wash.

20. Place the QIAamp 96 plate on top of a rack of elution microtubes making sure the tray of microtubes are oriented correctly with the QIAamp plate. Line the Biohazard waste container with several paper towels and empty filtrate from S-block into Biohazard waste container.

21. To elute the DNA, remove the tape and add 150uL Buffer AE to each well. Seal the QIAamp plate with an airpore tape sheet and incubate for 5 minutes at room temperature. Load the plate/elution microtube combination onto the centrifuge carrier, and then place it in the rotor bucket. Centrifuge at 6000 rpm for 4 minutes.

Note: If processing a partial plate, seal all used wells of the QIAamp 96 plate with tape and label them "used."

22. Cap the elution microtubes with the caps provided in the kit.

23. Discard the QIAamp 96 plate in the Biohazard trash. Remove the Biohazard waste from the Biosafety Cabinet, secure the bag with tape, and place in the large red Biohazard waste container.

24. Rinse S-blocks thoroughly in tap water, incubate for 1 minute at room temperature in 0.4 M HCl, empty the block, and wash thoroughly with distilled water before reusing. Used S-blocks can also be autoclaved after washing.

25. Store the extracted samples in a labeled elution tray as described below in "Comments on Storage" or continue on to PCR amplification.

Comments on Storage

Non-amplified DNA must be stored separately from amplified product. Store samples at 2-8°C.

Technical Assistance

For information and assistance regarding the performance or applications, contact QIAGEN Technical Service Department at 1-800-362-7737.

Reference

QIAamp 96 DNA Blood Kit - QIAGEN