|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| MP 1.01 Qiagen QIAamp DNA Mini Kit: Manual Blood Extraction | | | | | |
| **Purpose** | This procedure provides instructions for the rapid purification of total DNA from whole blood, plasma, and serum using a microcentrifuge. | | | | |
| **Policy Statements** | This procedure applies to technical staff performing testing on the QIAamp. | | | | |
| **Principle and Clinical Significance** | QIAamp DNA Blood Mini Kits provide fast and easy methods for purification of total DNA for reliable PCR. The simple QIAamp spin procedure is ideal for simultaneous processing of multiple samples and yields pure DNA ready for direct amplification. DNA is eluted in Buffer AE and ready for direct addition to PCR. Alternatively, it can be store at -30 to -15°C for later use. The purified DNA is free of protein, nucleases, and other contaminants or inhibitors.  DNA purified using QIAamp Kits is up to 50 kb in size, with fragments of approximately 20 – 30 kb predominating. DNA of this length completely denatures during thermal cycling and can be amplified with high efficiency. | | | | |
| **Sample** | 1. **Acceptable specimens: Fresh or Frozen**  * Whole blood – Lavender top, EDTA * Whole blood – Blue top, Sodium Citrate  1. **SDES codes/Specimen type:**  * Blood: BLD  1. **Specimen Collection and Transport:**  * Refer to [*Lab Test Directory*](http://starnet.childrenshc.org/departments-and-committees/lab-test-directory/) on StarNet  1. **Specimen assessment:**  * Refer to the policies MB 1.01 Specimen Management in Molecular Biology and MB 1.02 Specimen Rejection Criteria for Molecular Biology | | | | |
| **Special Safety Precautions** | * Do not perform assay in presence of vapors from sodium hypochlorite or dust. * Wear gloves at all times. * After all vortexing steps, briefly centrifuge the microcentrifuge tube to remove drops from inside the lid. * Change pipet tips between all liquid transfers and use aerosol-barrier pipet tips. * Open only one QIAamp Mini column at a time. Take care not to generate aerosols. * Discard filtrate and collection tubes in hazardous waste.   Molecular personnel are subject to occupational risks associated with specimen handling. Refer to the safety policies located in the safety section of the *Microbiology, Virology, and Molecular Procedure Manual:*   1. [*Safety in the Microbiology/Virology Laboratory*](file:///G:\Lab%20Procedures\Microbiology\1NEW%20Micro%20Procedure%20Manual.%20(same%20as%20in%20Starnet)\MCVI%203%20Safety\MCVI%203.2%20Safety%20in%20the%20Microbiology%20Lab.docx) 2. [*Safe Work Practices in Molecular*](https://starnet.childrenshc.org/References/labsop/molbio/safety/mb-2.01-safe-work-practices-in-molecular.pdf)  * [*Biohazardous Spills*](file:///G:\Lab%20Procedures\Microbiology\1NEW%20Micro%20Procedure%20Manual.%20(same%20as%20in%20Starnet)\MCVI%203%20Safety\MCVI%203.4%20Biohazardous%20Spills.docx) * [*Biohazardous Spill in Molecular*](https://starnet.childrenshc.org/References/labsop/molbio/safety/mb-2.03-biohazardous-spills-in-molecular.pdf) * *MB 2.02* [*Biohazard Containment*](https://starnet.childrenshc.org/References/labsop/index.php?view=folder&folder=molbio) * *MB 3.01 Engineering Controls to Prevent Nucleic Acid Contamination* | | | | |
| **Materials** | |  |  |  | | --- | --- | --- | | Reagents | Supplies | Equipment | | * 5% Extran * 70% Ethanol * Water * Sani-Cloth Bleach Wipes (10%) * Ethanol (96 – 100%) * RNase A Stock solution   Qiagen QIAamp DNA Mini Kit Reagents:   * Buffer AL * Buffer AW1 (concentrate) * Buffer AW2 (concentrate) * Buffer AE * QIAGEN Protease * Protease Solvent   QIAamp Buffers can be stored dry at room temperature (15 – 25 °C) for up to 1 year | * Lab coat * Paper towels/Kim wipe towels * Sharps container * Micro tubes 1.5 ml, RNase/DNase free * Pipette tips with aerosol barrier, 20 µl, 100 µl, 200 µl, 1000 µl * Nitrile gloves (powder-free) * Gripper rack, rm2 * Qiagen 2 mL Collection Tubes   Qiagen QIAamp DNA Mini Kit Supplies:   * QIAamp Mini Spin Columns * 2 mL Collection Tubes   QIAamp Mini spin columns can be stored dry at room temperature (15 – 25 °C) for up to 1 year | * Vortex * Pipets 20 µl, 100 µl, 200 µl, 1000 µl * Heating block at 56°C * BioSafety Cabinet * Microcentrifuge (with rotor for 2 ml tubes) * Thermometer | | | | | |
|
| **Procedure** | **Prepare for testing:**   1. Equilibrate Proteinase K to room temperature. 2. Mix Buffer AL thoroughly. Dissolve precipitates in Buffer AL by incubating at 56°C if necessary (set on top of heat block). 3. **NOTE:** This is stable for 1 year at room temperature. 4. Add the appropriate amount of ethanol to the Buffer AW1 as indicated on the bottle. 5. **NOTE:** This is stable for 1 year at room temperature. 6. Add the appropriate amount of ethanol to the Buffer AW2 as indicated on the bottle. 7. **NOTE:** This is stable for 1 year at room temperature. 8. Set heat block to 56°C. 9. Bring samples to room temperature (15 – 25°C). If sample is frozen, allow blood to thaw completely to room temperature. 10. Equilibrate Buffer AE to room temperature for elution.   **DNA Purification:**   1. Pipet 20 μl Proteinase K into the bottom of a labeled 1.5 ml microcentrifuge tube. 2. Invert sample 5 – 7 times and add 200 μl of the sample to the microcentrifuge tube. If sample is larger than 200 μl, increase the amount of Proteinase K and AL proportionally (see Step 3 for example). 200 μl of blood yields 3 – 12 μg of DNA. 3. If RNA-free genomic DNA is required add 4 μl of RNAase A stock solution (10 mg/mL) to the sample before addition of Buffer AL. 4. Add 200 μl Buffer AL to the sample. Do NOT add Proteinase K directly to Buffer AL. 5. If sample was greater than 200 μl increase proportionally. Example: 400 μl sample requires 40 μl Proteinase K and 400 μl Buffer AL. 6. Pulse-vortex for 15 seconds. 7. Incubate at 56°C in heat block for 10 minutes. 8. **NOTE:** Longer incubation times will have no effect on yield or quality of purified DNA. 9. Briefly centrifuge the 1.5 ml microcentrifuge tube. 10. Add 200 μl ethanol and mix again by pulse-vortexing for 15 seconds. 11. **NOTE:** If sample was greater than 200 μl then increase ethanol proportionally. 12. Briefly centrifuge (quick-spin). 13. Apply mixture to a labeled QIAamp Mini spin column in a labeled 2 ml collection tube without wetting the rim. 14. Close the cap and centrifuge for 1 minute at 8000 rpm. 15. Place QIAamp Mini spin column in a labeled clean 2 ml collection tube and discard the tube containing the filtrate. 16. Carefully open the QIAamp Mini spin column and add 500 μl Buffer AW1 without wetting the rim. 17. **NOTE:** Do NOT increase the volume of AW1 if the original sample was greater than 200 μl. 18. Close the cap and centrifuge for 1 minute at 8000 rpm. 19. Place QIAamp Mini spin column in a labeled clean 2 ml collection tube and discard the tube containing the filtrate. 20. Carefully open the QIAamp Mini spin column and add 500 μl Buffer AW2 without wetting the rim. 21. Close the cap and centrifuge at 14,000 rpm for 3 minutes. 22. Place QIAamp Mini spin column in a labeled new 2 ml collection tube and discard the old collection tube with the filtrate. 23. Centrifuge at 14,000 rpm for 1 minute. 24. Place QIAamp Mini spin column in a new labeled 2 ml collection tube and discard the collection tube containing the filtrate. 25. Carefully open the QIAamp Mini spin column and add 200 μl Buffer AE. 26. Incubate at room temperature for 5 minutes. 27. Centrifuge at 8000 rpm for 1 minute.   **NOTE:** for DNA quantitation use Buffer AE as a blank. | | | | |
| **References** | 1. QIAamp DNA Mini and Blood Mini Handbook, Fifth Edition, May 2016. | | | | |
| **Training Plan/ Competency Assessment** | **Training Plan** | | | **Initial Competency Assessment** | |
| 1. Employee must read the procedure. 2. Employee will demonstrate the ability to perform procedure, record results, and document corrective action after instruction by the trainer. | | | 1. Direct observation | |
| **Historical Record** |  |  |  | |  |
|  | **Version** | **Written/Revised by:** | **Effective Date:** | | **Summary of Revisions** |
| 1 | Michelle Merryman / Julie Laramie | 4/20/2020 | | Initial Version |
|  |  |  | |  |
|  |  |  | |  |
|  |  |  |  | |  |  |  |
|  |  |  | |  |
| **Archived by:** |  | **Archived Date:** | |  |