DNA Isolation with Qiagen QIAamp DNA Mini Kit

This protocol applies to: Acute Lymphoblastic Leukemia Phase I (ALL P1), Acute Lymphoblastic Leukemia Phase 2 (ALL P2), and Acute Lymphoblastic Leukemia Models (ALL MDLS/Xenografts)

The protocol herein describes the procedures used by Nationwide Children's Hospital to process disease tissues for RNA and/or DNA subsequently used for characterization in the NCI's TARGET initiative. All nucleic acid samples used in TARGET projects were quality tested for consistency using picogreen quantification and SSTR genotyping methods, regardless of where the nucleic acid was originally extracted.

I. PRINCIPLE

The QIAamp DNA Mini kit provides a fast and easy method for the purification of DNA from lymphocytes for reliable PCR and Southern Blotting. The QIAamp spin procedure uses a special lysate buffering condition that allows optimal binding of the DNA to the QIAamp membrane upon centrifugation. Salt and pH conditions in the lysate ensure that protein and other contaminants, which can inhibit PCR, are not retained on the QIAamp membrane. The bound DNA is washed in two centrifugation steps to ensure complete removal of any residual contaminants without affecting DNA binding and the purified DNA is eluted from the QIAamp spin column using a buffered solution.

II. SPECIMEN

A. Types

White blood cell pellet suspended in Hank's balanced salt solution (HBSS).

B. Handling Conditions

Follow standard precautions when handling all cell suspensions.

C. Sample Preparation

Peripheral blood and bone marrow samples are ficolled or lysed to obtain white blood cells. The cells are washed with Hank's Balanced Salt Solution and centrifuged to pellet.

D. Indications for Study

DNA is isolated from white blood cells for any DNA-based molecular testing performed in the laboratory. This protocol is used most frequently for ALL Day 28 peripheral blood specimens after the ficoll separation of white blood cells or lysing of red blood cells.

III. REQUIRED EQUIPMENT, SUPPLIES AND REAGENTS

A. Equipment

Eppendorf centrifuge 5810R with 15mL and 50mL tube holders
Eppendorf 5415R microcentrifuge
Capsule centrifuge
Pipetters-Adjustable, 1-10µL, 2-20µL, 20-200µL, 100-1000µL
Vortex mixer
Water bath at 56˚C

B. Supplies

Aerosol barrier pipet tips
15 mL Polypropylene tubes
50 mL Polypropylene tubes
1.5 mL microcentrifuge tubes
Microcentrifuge tube rack
Racks to hold 15 mL and 50 mL polypropylene tubes
Permanent-ink colored markers
PPE (Lab coat, Gloves)
TEC label, 2" x 0.625" (AIDC Solutions, Catalog# TTLPC-2-0625-1-5000-1)
Brady FreezerBondz Label, 1" x 05" (Fisher, Catalog# 22-500-520)

C. Reagents

Hank's balanced salt solution without phenol red (Sigma, Catalog# H8264)
Ethanol (Sigma-Aldrich, Catalog# E7023)
QIAamp DNA Mini Kit 250 (Qiagen, Catalog# 51306)

IV. WARNINGS / PRECAUTIONS

A. Use Standard Precautions when handling all body fluids, tissues and cell cultures. Refer to the Specimen Handling and Collection procedure, GEN-1, for guidelines specific for the Molecular Genetics Laboratory and samples.

B. Buffers AL and AW1 contain guanidine hydrochloride, which can form highly-reactive compounds when combined with bleach. To clean a spill with these

chemicals, use a lab detergent and water; to decontaminate, follow with sodium hypochlorite instead of bleach.

V. REAGENT PREPARATION (INCLUDING STORAGE CONDITIONS)

A. Hank's balanced salt solution and the QIAamp DNA Mini Kit are stored at room temperature and used before the manufacturer's expiration date.

B. Before using new kit reagents, add the appropriate amount of 100% ethanol if applicable (Buffers AW1 and AW2).

C. Ethanol is stored at room temperature in a flammables chemical cabinet.

VI. QUALITY CONTROL

A. Specimen identification is assured through each phase of handling by assignment of a specific color of tape to each sample. Place a piece of the colored tape (in addition to the two identifiers for the sample) on each tube used throughout the procedure.

B. At each step in the isolation, the supernatant or pellet that does not contain the DNA is retained until after isolation and quantitation is completed. All tubes to be discarded are labeled with at least two identifiers and the step of the procedure from which it was used.

C. All new lots of reagents are tested in parallel with the one in current use before being put into clinical use; results are recorded in the QC book or in-lab QC sheet. All kit components must be quality control tested and used together thereafter. All reagents supplied in a kit must be used only with other reagents in the same kit lot number; reagents with identical lot numbers cannot be used interchangeably between kit lot numbers.

VII. PROCEDURE – STEPWISE

A. Sample Preparation

1. Label appropriate tubes for processing samples with the specific color marker, patient initials, registration number, specimen type, the last 3 digits of the USI for ALL specimens, and isolation step. Place a similar label on the original aliquot tube. Label the mini column with 2 identifiers and colored marker.

2. Determine the total cell count (3.0×10^6 – 1.0×10^7) using the Cell Counting and Viability procedure, REF-4. For thawed, banked specimens,

the cell count on the vial is used. Aliquot the volume in an appropriate sized tube.

3. Centrifuge aliquot of cells for 7 minutes at 400 x g with brake. Pour off supernatant and resuspend cells, with Hank's balanced salt solution, in a total volume of 200 µL. (Note: may freeze pellet in a -80˚C freezer for up to a week)

B. Cell Lysis

1. Pipet 20 µL of Qiagen Proteinase K into the bottom of a 1.5 mL microcentrifuge tube.
2. Add the 200 µL cell suspension (3.0 X 10^6-1.0 X 10^7 total cells) to the Proteinase K.
3. Add 200 µL of Buffer AL to the cell suspension/Proteinase K and vortex for 15 seconds.
4. Incubate in a 56˚C water bath for 10 minutes. Longer incubation times have no effect on yield or quality of the purified DNA.
5. Briefly centrifuge to remove any droplets that may have formed at the top.

C. Adsorption of DNA to the QIAamp Column

1. Add 200 µL of 100% ethanol and mix by vortexing for 15 seconds.
2. Briefly centrifuge and add the entire mixture to a QIAamp spin column. Centrifuge at 13,200 rpm in the Eppendorf 5415R microcentifuge for 1 minute at room temperature.

D. Removal of Residual Contaminants

1. Remove the spin column and place in a clean labeled collection tube.
2. Add 500 µL of buffer AW1 and centrifuge at 10,000 rpm in the Eppendorf 5415R microcentifuge for 1 minute at room temperature.
3. Remove the spin column and place in another clean labeled collection tube.
4. Add 500 µL of Buffer AW2 and centrifuge at 13,200 rpm in the Eppendorf 5415R microcentifuge for 3 minutes at room temperature.

E. Eluting DNA and Storage

1. Remove the spin column and place in a clean, 1.5 mL microcentrifuge tube that is labeled with the DNA number, Registration number, patient

initials and the date of isolation; label the lid of the tube with the DNA number and date isolated. Create a label from STARS and label with the registration number.

2. Determine elutions based on cell count of the sample according to the following chart:

Cell Count	1st Elution	2nd Elution (fresh)	Total Elution Volume
$< 1 \times 10^6$	25 µL	25 µL	50 µL
$1 \times 10^6 - 4 \times 10^6$	50 µL	50 µL	100 µL
$5 \times 10^6 - 1 \times 10^7$	100 µL	100 µL	200 µL

3. Carefully open the QIAamp Mini spin column and add the appropriate volume of Buffer AE. Incubate at room temperature for 5 minutes and centrifuge at 6,000 x g (8,000 rpm) for 1 minute. Repeat this step again for the second elution volume.

    a. *NOTE*: Incubating the QIAamp Mini spin column loaded with Buffer AE for 5 minutes at room temperature before centrifugation generally increases DNA yield. A second elution step with further Buffer AE will increase yields by up to 15%.

    b. *NOTE*: Centrifugation of QIAamp Mini columns is performed at 6,000 x g (8,000 rpm) in order to reduce centrifuge noise. Centrifuging QIAamp Mini columns at full speed will not affect DNA yield. Centrifugation at a lower speed is also acceptable, provided that nearly all of each solution is transferred through the QIAamp membrane. When preparing DNA from buffy coat or lymphocytes, full-speed centrifugation is recommended to avoid clogging. All centrifugation steps should be carried out at room temperature.

4. Quantitate DNA by using PicoGreen (refer to GEN-9).

5. A 100 ng/µL dilution is made for all DNA samples (except in cases where elution volume and concentrations are low). If the concentration is <100 ng/µL for high volume samples, concentrate the sample by drying it down until a higher concentration is observed.

    a. Maxi = 200 µL of 100 ng/µL

    b. Midi = 100 µL of 100 ng/µL

c. Mini= 50 µL or 100µL of 100 ng/µL

REMEMBER: $C^1 \times V^1 = C^2 \times V^2$

6. Store DNA in a -20˚C or -80˚C freezer indefinitely.

VIII. INTERPRETATION / ANALYSIS / DOCUMENTATION

A. For each sample, the following information is recorded onto a DNA worksheet that is later recorded in STARS: sample volume, resuspension volume, dilution factor, absorbance ratio, concentration and yield.

B. DNA yields from white blood cells are expected to be up to 50 µg from 200 µL of 3.0×10^6 - 1.0×10^7 cells used.

IX. REFERENCES

1. QIAamp DNA Mini Kit: For DNA Purification from Lymphocytes. June 2012.