Title: Purification of DNA and Viral RNA Using QIAamp Mini Kits

SOP Number: 23113 **Revision Number: 02**

Supersedes: Revision 01 **Effective Date: JUL 26 2011**

[redacted]

Table of Contents

1.0 Purpose

This SOP will provide methods for purification of total DNA as well as RNA, including viral RNA that is suitable for PCR.

This procedure is made available through federal funds from the National Cancer Institute, NIH, under contract HHSN261200800001E.

2.0 Scope

This SOP refers to various QIAamp Mini Kits. The limit to DNA size for which these kits may be used for purification is 50 kb, with fragments of approximately 20–30 kb predominating. The maximum DNA content for which this SOP is appropriate is an average of 6 µg of total DNA/RNA (e.g., genomic, viral, mitochondrial) from 250 µg DNA per milliliter of solution OR 5 x 10^6 lymphocytes/cultured cells that have a normal set of chromosomes. This procedure will be performed by trained PA personnel.

Note: All infectious bacterial or viral samples must be handled in accordance with **SOP 26101*, Labeling, Transport, Submission, Storage, and Handling of Biohazardous Materials within the BDP***.

3.0 Authority and Responsibility

3.1 The Director, Process Analytics (PA) has the authority to define this procedure.

3.2 PA is responsible for training laboratory personnel and documenting this training to Biopharmaceutical Quality Assurance (BQA).

3.3 PA personnel are responsible for the performance of this procedure.

3.4 PA is responsible for reviewing the data and documentation of the results of this procedure.

3.5 BQA is responsible for quality oversight of this operation.

4.0 Materials, Equipment and Reagents

4.1 Biosafety cabinet (BSC).

4.2 Bench-top, high-speed centrifuge.

4.3 Tube racks.

4.4 1.5 mL DNase-free and RNase-free microcentrifuge tubes (BDP PN 20394), or BDP approved equivalent.

4.5 15 mL centrifuge tube (BDP PN 20006), or BDP approved equivalent.

4.6 Disposable Serological Pipets: 10 mL (BDP PN 20100), 25 mL (BDP PN 20102), 50 mL (BDP PN 20105), or BDP approved equivalent.

4.7 Pipettes: 10 µL, 20 µL, 100 µL, 200 µL, or 1000 µL, or BDP approved equivalent.

4.8 Inoculating loop (BDP PN 20505), or BDP approved equivalent.

4.9 Sterile, RNase-free disposable pipette tips with Aerosol Barriers: 0-10 µL (BDP PN 20335), 10-100 µL, (BDP PN 21484), 200 µL, (BDP PN 20673), 1000 µL, (BDP PN 20769), or BDP approved equivalent.

4.10 Pipet-Aid pipettor, or BDP approved equivalent.

4.11 Water baths or heating blocks.

4.12 Thermometer.

4.13 Disposable gloves (BDP approved).

4.14 DEPC Treated Water, Nuclease-free, (BDP PN 30266) or approved equivalent.

4.15 PBS, pH7.4 (BDP PN 30007).

4.16 Ethanol (BDP PN 10106), or BDP approved equivalent.

4.17 RNase A (BDP PN 10265), or BDP approved equivalent.

4.18 Qiagen QIAamp® DNA Mini Kit (BDP PN 30442)* for purification of DNA from plasma, serum, fluids, cultured cells (5 x 10^6 diploid per 200 µL PBS).

4.19 Qiagen QIAamp® Viral RNA Kit (BDP PN 30450).*

4.20 Qiagen QIAamp® DNA Blood Mini Kit (BDP PN 30444).*

Components	QIAamp® Mini Kits		
	DNA	DNA Blood	Viral RNA
	PN 30442	PN 30444	PN 30450
Proteinase K	+++	None	None
Qiagen Protease	None	+++	None
Protease Solvent	None	+++	None
Carrier RNA (poly A)	None	None	+++
Lysis Buffer	Buffer AL or ATL	Buffer AL	⁂ Buffer AVL
Spin Columns	+++	+++	+++
Collection Tubes (2 mL)	+++	+++	+++
1st Wash Buffer	Buffer AW1	Buffer AW1	Buffer AW1
2nd Wash Buffer	Buffer AW2	Buffer AW2	Buffer AW2
Elution Buffer	Buffer AE	Buffer AE	Buffer AVE

Table 1: Contents of QIAamp Mini Kits
⁂ Inactivates potentially infectious agents & RNases
+++ Components present in the Mini Kit.

5.0 Procedural Criteria

Refer to Attachment 2 for a pictorial summary of the purification process.

5.1 If the sample is required to undergo fluorometric analysis for dsDNA quantitation subsequent to purification, a minimum of 1 mL should be provided for extraction.

5.2 If the sample is potentially infectious, handle per **SOP 22923, *Procedures for Safe Handling and Decontamination of Viruses by BDP/BPA and Related Personnel***. Viral samples can be inactivated by the requestor in a BSC prior to delivery to PA Accessioning per **SOP 22005, *Viral Inactivation Procedures with Guanidinium Buffers***. Alternatively, PA staff can inactivate appropriately-labeled infectious samples per **SOP 22005*, Viral Inactivation Procedures with Guanidinium Buffers***. PA staff will follow Step 6.0 using the QIAamp Mini Kit of this SOP.

5.3 To purify genomic DNA from proteinaceous aqueous solutions, cultured cells or lymphocytes in PBS, serum, buffy coat, and body fluid samples, follow the procedure outlined in Step 6.0. Use this procedure, and the QIAamp Mini Kit if the samples have > 8 mM EDTA content.

5.4 To purify genomic DNA from whole blood treated with citrate, heparin or EDTA, from body fluids or from animal cell culture, follow Step 6.0 for procedure using the QIAamp Blood Mini Kit (BDP PN 30444.)

5.5 To purify DNA from solid tissue, bacteria (gram-negative) using the QIAamp Mini Kit, follow Step 7.0.

5.6 Purify Viral RNA, greater than 200 nucleotides in length from cell-free fluids using the Viral RNA Mini Kit (BDP PN 30450) and step 8.0.

6.0 Purification of DNA (QIAamp Mini Kit or QIAamp Blood Mini Kit)

6.1 Reagent Preparation

6.1.1 QIAGEN Protease (when using the QIAamp Blood Mini Kit): Pipet 5.5 mL protease solvent into the vial containing lyophilized QIAGEN Protease. Indicate reconstitution of the material on the label of vial. The dissolved QIAGEN protease is stable for up to two (2) months at 2-8°C. Aliquots may be stored at -20°C and are stable for up to a year. Label the dissolved QIAGEN protease per **SOP 22702, *Solutions Used in Process Analytics,*** including the appropriate expiration date.

6.1.2 Buffer AL or ATL: Mix thoroughly by shaking before use. If a precipitate is present, dissolve by incubating in a 56 ± 4°C water bath for 7 ± 3 minutes. Buffer AL or ATL is stable for one (1) year when stored closed at room temperature (15-25°C).

6.1.3 Buffer AW1: Add 125 mL Ethanol (96-100%) to the container of concentrated Buffer AW1. Indicate the date of reconstitution on the provided label of the container. Buffer AW1 is stable for one (1) year when stored closed at room temperature (15-25°C).

6.1.4 Buffer AW2: Add 160 mL Ethanol (96-100%) to the container of concentrated Buffer AW2. Indicate reconstitution on the provided label of the container. Buffer AW2 is stable for one (1) year when stored closed at room temperature (15-25°C).

<u>Purification of a 200 µL Sample</u>

6.1.5 Heat water bath to 56 ± 4°C.

6.1.6 Equilibrate the sample to room temperature (15 - 25°C).

6.1.7 Equilibrate all buffers to room temperature.

6.1.8 Confirm that all buffers have been appropriately prepared.

6.1.9 Pipet 20 µL Qiagen Protease (or Proteinase K) into the bottom of a 1.5 mL microcentrifuge tube. Should the starting volume be greater than 200 µL, use a 15 mL centrifuge tube at this juncture or multiple 1.5 mL microcentrifuge tubes, and increase the volume of enzyme in proportion to that of the sample volume.

6.1.10 Add a 200 µL sample, or up to 5 x 10^6 cells (pelleted by 5-minute centrifugation at 300 x g) resuspended in 200 µL PBS to the microcentrifuge tube. Bring sample volume to 200 µL with PBS if its starting volume is less than 200 µL.

6.1.10.1 If a sample is going to be used for fluorometric analysis, purify at least 400 µL of the sample, processing aliquots uniformly.

6.1.10.2 If the RNA-free genomic DNA is required, add 4 µL of RNase A stock solution, 100 mg/mL (BDP PN 10265).

6.1.11 Mix thoroughly by pulse-vortexing for 15 seconds.

6.1.12 Add 200 µL **Buffer AL** to the sample. Mix thoroughly by pulse-vortexing for 15-20 seconds. If the starting volume is greater than 200 µL, increase the volume of **Buffer AL** proportionally.

6.1.13 Incubate the mixture in a water bath or heating block at 56 ± 4ºC for 10-12 minutes.

6.1.14 Centrifuge for 30 ± 5 seconds at 6000 x *g* (8000 rpm) to remove drops from the lid. Perform this, and all centrifugations, at room temperature.

6.1.15 Add 200 µL or proportional volume of ethanol to the sample and mix by pulse-vortexing for 15-20 seconds.

6.1.16 Centrifuge 30 ± 5 seconds at 6000 x *g* (8000 rpm) to remove drops from the lid.

6.1.17 Carefully apply the mixture (620 µL) to the QIAamp Spin column which has been inserted in a 2 mL collection tube. Avoid wetting the rim during application.

6.1.18 Centrifuge the covered Spin Column assembly. Make 1-minute increments in centrifugation time until the column is empty. (If purifying DNA from lymphocytes or buffy coat, centrifuge at 20000 x *g* or 14000 rpm).

6.1.19 Place the Spin Column in a clean 2 mL collection tube. Apply 500 µL Buffer AW1 (reconstituted) to the column without wetting the rim. Cap the column and centrifuge at parameters set to 6000 x *g* (8000 rpm), and one (1) minute.

6.1.20 Place the Spin Column in a clean 2 mL collection. Apply 500 µL Buffer AW2 (reconstituted) to the column without wetting the rim. Cap the column and centrifuge at parameters set to 20,000 x *g* (14,000 rpm), and four (4) minutes.

6.1.21 Place the QIAamp Spin Column in a clean, appropriately labeled microcentrifuge tube. Add 150– 200 µL **Buffer AE** to the Spin Column.

6.1.22 Allow the column to incubate for 5 – 10 minutes at room temperature, then elute the purified DNA by centrifugation at parameters set to 6000 x *g* (8000 rpm) and 1 minute.

6.1.23 The purified DNA may be stored at 2-8°C, or for extended periods at -20°C.

7.0 Purification of DNA from Tissue (QIAamp Mini Kit)

Note: The maximum starting sample size is 25 mg (10 mg, if of spleen origin) tissue in powder form, and 1 mL plated or suspension bacterial cultures.

7.1 Prepare reagents as in Step 6.1.

7.1.1 If the sample is low copy (≤ 10,000 copies present), prepare a vial of Carrier RNA (Poly A) in solution of BDP PN 30450 by adding 1 mL of Buffer AVL or ATL (lysis buffer) to the vial of lyophilized Poly A. Use this instead of absolute Buffer ATL.

7.2 Heat two water baths or heating blocks: one at 56 ± 4ºC and the other 70 ± 2°C.

7.3 Place tissue in a micocentrifuge tube and add 180 µL Buffer **ATL** (for plated bacterial culture, transfer to Buffer **ATL** with an inoculating loop). If purifying a Suspension culture, centrifuge 1 mL for 10 minutes at 5000 x *g* or 7500 rpm in a 1.5 mL microcentrifuge tube before bringing the volume to 180 µL with PBS.

7.4 Add 20 µL Proteinase K to the tube, mix by vortexing, then incubate at 56 ± 4ºC, 1-3 hours or overnight, until the tissue is completely lysed.

Note: Overnight lysis does not impact the process negatively.

7.5 During incubation, vortex for 15-20 seconds at regular intervals 2-3 times per hour, or place on a rocking platform.

7.6 At the end of incubation, centrifuge the tube 30-35 seconds to remove moisture from the lid.

7.7 If RNA-free DNA is NOT REQUIRED, this step may be skipped. Proceed to Step 7.8. **If RNA free genomic DNA is required**, add 4 µL RNase A (BDP PN 10265) to the tube.

7.7.1 Pulse-vortex for 15-20 seconds then incubate at room temperature for 2 -3 minutes.

7.7.2 Centrifuge the tube for 30-35 seconds at 6000 x *g* or 8000 rpm before performing Step 7.8.

7.8 **If RNA-free genomic DNA is not required**, add 200 µL Buffer **AL** to the sample.

7.8.1 Mix by pulse-vortexing for 15-20 seconds.

7.8.2 Incubate for 10-12 minutes at 70 ± 2°C.

7.8.3 Mix again by pulse vortexing for 15-20 seconds.

7.8.4 Centrifuge the tube for 30 seconds at 6000 x *g* or 8000 rpm to remove droplets from the lid.

7.9 Add 200 µL of 96-100% (230 µL for low-copy sample) ethanol to the sample, and mix thoroughly by pulse-vortexing for 15-20 seconds.

7.9.1 Centrifuge the tube at parameters set to 30 seconds at 6000 x *g* or 8000 rpm.

This procedure is made available through federal funds from the National Cancer Institute, NIH, under contract HHSN261200800001E.

7.10 Apply the entire volume of mixture, including any precipitate, to the QIAamp Spin Column Assembly and perform Steps 6.2.14 through 6.2.18.

7.11 Repeat Steps 6.2.17 through 6.2.18 twice. **Exception:** For low-copy samples, elute with 60 µL of **Buffer AE** only.

7.12 The purified DNA may be stored overnight at 2-8°C, or for extended periods at ≤ -20ºC.

8.0 Purification of Viral RNA Using QIAamp Viral RNA Mini Kit (140 µL Sample Size)

WARNING: Perform all processes with viral samples in compliance with SOP 22923, *Procedure for Safe Handling and Decontamination of Viruses by BDP/BPA and Related Personnel*.

SAFETY NOTE: Before beginning extraction of the Viral RNA from samples previously inactivated by a requestor, perform a second viral inactivation per SOP 22005*, Viral Inactivation Procedures with Guanidinium Buffers* in a Biological Safety Cabinet.

8.1 Sample and Reagent Preparation

8.1.1 Equilibrate all samples to room temperature (15-25°C).

8.1.2 Prior to submitting the request to PA for purification of the sample, the requestor must perform the following.

8.1.2.1 If necessary, adjust the sample volume to 140 µL with PBS if starting material is less than 140 µL. If samples are greater than 140 µL and of low viral titer, 3.0 ± 0.5 mL cell-free material should be concentrated to 140 µL or less.

8.1.2.2 The requestor or PA staff must inactivate the virus per **SOP 22005, *Viral Inactivation Procedures with Guanidinium Buffers*,** and complete and submit a copy of the completed Form 22005-01, Part A. PA will complete Part B of Form 22005-01 as necessary.

8.1.3 Check **Buffer AVL** for precipitation, and if necessary incubate in an 80 ± 2°C water bath for no more than 5 minutes to dissolve the precipitate. Allow the buffer to cool to room temperature before use. The AVL Buffer may not be warmed more than six (6) times.

8.1.3.1 If using Buffer AVL for the first time, add 1 mL of Buffer AVL to one tube of lyophilized Carrier RNA. Dissolve the Carrier RNA thoroughly. Transfer the mixture to the Buffer AVL bottle, and mix thoroughly before use. This may be stored at 2-8°C for up to 6 months. Indicate the Preparation and Expiry dates on the bottle label.

8.1.4 Reconstitute **Buffer AW1** and **AW2** as in Steps 6.1.3 and 6.1.4 respectively.

8.1.5 Equilibrate **Buffer AVE** to room temperature, if necessary.

8.1.6 Confirm that the sample was inactivated per **SOP 22005, *Viral Inactivation Procedures with Guanidinium Buffers***. The initial inactivation may be performed by the Test Requestor or by PA staff. Check for a copy of Form 22005-01 with the pertinent information.

8.2 Viral RNA Purification Procedure

8.2.1 In the BSC, dispense a volume of prepared **Buffer AVL/Carrier RNA** mixture that is equivalent to the starting volume of the Viral RNA sample in the tube containing the inactivated test sample mixture. Mix by gentle vortexing for at least 15 seconds until a homogeneous solution is formed.

8.2.2 Incubate the mixture at room temperature for at least 10 minutes to ascertain complete lysis/inactivation of the virus.

8.2.3 Centrifuge the samples 20-30 seconds at 6000 x *g* or 8000 rpm to remove drops from the inside of lids of the centrifuge tubes.

8.2.4 Add ethanol to the sample equivalent to four times (4X) the starting volume. Vortex for 15-20 seconds to mix, then centrifuge for 20-30 seconds with parameters set to 6000 x *g* (8000 rpm).

8.2.5 Apply 600 µL of the solution to a QiaAmp spin column placed in a 2 mL collection tube. Take care to avoid wetting rim. Close the cap.

8.2.6 Centrifuge with parameters set at 6000 *x g* (8000 rpm) and 1 minute. Discard the tube containing the filtrate.

8.2.7 Repeat Steps 8.2.5 and 8.2.6 using a second (clean) 2 mL collection tube. If the sample is greater than 140 µL, repeat this step until all the lysate has been loaded onto the column.

8.2.8 Place the QIAamp spin column in a clean 2 mL collection tube. Open the spin column and add 500 µL Buffer AW1 to it. Multiple washes with AW1 are not necessary when the initial sample volume is greater than 140 µL.

8.2.9 Repeat step 8.2.6.

8.2.10 Place the QIAamp spin column in a clean 2 mL collection tube; open the spin column and add 500 µL of Buffer AW2.

8.2.11 Close the column and centrifuge with parameters set at 20000 *x g* or 14000 rpm, and 3 minutes.

8.2.12 Place the QIAamp spin column in a clean collection tube and centrifuge at 20000 *x g* (14000 rpm) for an additional minute to remove any residual wash buffer, then discard.

8.2.13 Place the QIAamp spin column in a clean microcentrifuge tube. Add 40 µL of room temperature Buffer AVE to the membrane of the QIAamp spin column to elute the Viral RNA. Take care to not touch the surface of membrane with the pipette tip.

8.2.14 Close the cap of the spin column and allow the buffer to incubate at room temperature for 60-70 seconds.

8.2.15 Centrifuge at settings of 1 minute and 6000 *x g* or 8000 rpm.

8.2.16 Add a second 40 µL volume of Buffer AVE or RNase-free water to the membrane and repeat steps 8.2.14 – 8.2.15.

8.2.16.1 A single elution of Viral RNA with 60 µL Buffer AVE or RNase-free water may be performed as an alternative to two (2) 40 µL elutions.

8.2.17 If samples will be processed or analyzed within 24 hours, store at 2-8°C. Store remaining sample or aliquots of the sample for up to one (1) year at ≤ -20°C. Ensure that samples/aliquots are labeled with sample name, lot number, preparation date, expiry and storage temperature.

9.0 Analysis of Purified Samples

9.1 Quantitate the DNA Extract using A_{260} per **SOP 22158*, Operation of the Beckman DU Series 600 Spectrophotometer***, or **SOP 22941*, Operation of the Beckman Coulter DU800 Spectrophotometer,*** or by PicoGreen per **SOP 22156*, PicoGreen dsDNA Quantitation Assay.*** Data must be captured on forms pertaining to the respective Standard Operating Procedures utilized for DNA quantitation.

9.1.1 The use of the NanoDrop ND-1000 spectrophotometer is advised for in-process DNA quantitation (i.e., the precursor to agarose gel electrophoresis) to minimize sample loss. See **SOP 22946*, The Operation of the NanoDrop ND-1000 Spectrophotometer.***

9.2 Where required, follow **SOP 22149, *Restriction Endonuclease Enzyme Digestion of Plasmid DNA**, and* SOP 22148*, Agarose Gel Electrophoresis (A.G.E.) and Detection of Nucleic Acids,*** for enzyme digestion, preparation of agarose gels, and for general electrophoretic conditions. Pertinent information may be captured on Form 22148-01 and/or 22149-01.

9.2.1 Perform gel imaging and analysis of the agarose gel per **SOP 22120, *Digital Gel Imaging Using the Kodak 400 Image Station***.

9.3 Samples may be analyzed, if required, per **SOP 22195, *Quantitative PCR (qPCR) Methods for Detection and Quantitation of Nucleic Acids***. Pertinent data for this process must be captured on Forms 22195-01 through 22195-03.

10.0 Documentation

10.1 Attach all forms pertaining to the DNA/viral RNA purification process.

10.2 Attach all forms indicating inactivation of viral particles where appropriate.

10.3 All solutions are prepared according to **SOP 22702, *Solutions Used in Process Analytics***.

10.4 Documents pertaining to this procedure shall be handled and retained per **SOP 21409, *Good Documentation Practices***.

11.0 References and Related Documents

11.1 **SOP 21409** *Good Documentation Practices*

11.2 **SOP 22005** *Viral Inactivation Procedures with Guanidinium Buffers*

11.3 **SOP 22120** *Digital Gel Imagining Using the Kodak 400 Image Station*

11.4 **SOP 22148** *Agarose Gel Electrophoresis (A.G.E.) and Detection of Nucleic Acids*

11.5 **SOP 22149** *Restriction Endonuclease Enzyme Digestion of Plasmid DNA*

11.6 **SOP 22156** *PicoGreen dsDNA Quantitation Assay*

11.7 **SOP 22158** *Operation of the Beckman DU Series 600 Spectrophotometer*

11.8 **SOP 22195** *Quantitative PCR (qPCR) Methods for Detection and Quantitation of Nucleic Acids*

11.9 **SOP 22702** *Solutions Used in Process Analytics*

11.10 **SOP 22923** *Procedures for Safe Handling and Decontamination of Viruses by BDP/BPA and Related Personnel*

11.11 **SOP 22941** *Operation of the Beckman Coulter DU800 Spectrophotometer*

11.12 **SOP 22946** *The Operation of the NanoDrop ND-1000 Spectrophotometer*

11.13 **SOP 26101** *Labeling, Transport, Submission, Storage, and Handling of Biohazardous Materials within the BDP*

11.14 QIAGEN QIAamp DNA Mini Kit and QIAamp DNA Blood Mini Kit Handbook, February 2003.

11.15 QIAGEN QIAamp Viral RNA Mini Kit Handbook, January 1999.

12.0 Attachments

12.1 **Attachment 1** Form 23113-01, Sample Preparation Equipment, Reagents, Materials

12.2 **Attachment 2** Form 23113-02, Summary of QIAamp Mini Spin Procedure

This procedure is made available through federal funds from the National Cancer Institute, NIH, under contract HHSN261200800001E.

Attachment 1

NCI-Frederick
Form No.: 23113-01
SOP No.: 23113
Revision 02:

Sample Preparation Equipment, Reagents, Materials

QC#_____

Materials/Reagents

Reagents	BDP PN	BDP Lot No.	Expiration Date
QiAmp DNA Kit (Circle one) Mini / Blood / Viral RNA			
Ethyl Alcohol			
PBS			
Additional Reagents:			

Equipment:

Description	MEF No./Serial No.	Calibration Due Date
Water Bath/Heat block		
Thermometer		
Micro-centrifuge		
Additional Equipment:		

Pipettors used _____

Sample Volume _____uL Lysis Buffer Type _____ Volume _____uL

Lysis Incubation Temperature:_____°C Lysis Time In:_____ Time out:_____

Elution Buffer:_____ Elution Volume:_____

Extraction Performed by: _____Date: _____

Reviewed By:_____ Date: _____

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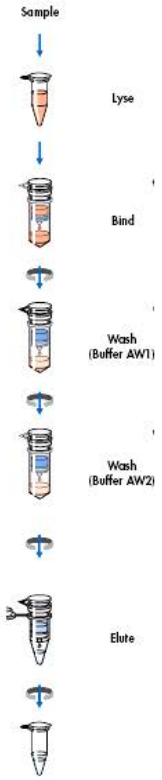
Attachment 2

NCI-Frederick
Form No.: 23113-02
SOP No.: 23113
Revision 02:

Summary of QIAamp Mini Spin Procedure



Pure DNA or Viral RNA