INTEGRATED BIOREPOSITORY OF H3AFRICA UGANDA

MAKERERE UNIVERSITY
COLLEGE OF HEALTH SCIENCES

STANDARD OPERATING PROCEDURE

TITLE: **PAXGENE BLOOD RNA ISOLATION**		**PAGE 1 of 11**
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VALIDATION AND RETIREMENT

	NAME	DATE
Validated by:		
Retired by:		

ACKNOWLEDGEMENT OF READING AND UNDERSTANDING

I have received and understood the training on this SOP. If I have not understood the training I have asked the trainer to retrain me to ensure that I completely understand all the requirements.

	NAME	SIGNATURE	DATE
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1 PURPOSE

To describe the procedures required to isolate high quality RNA from human blood collected in PAXgene blood RNA tubes.

2 SCOPE

This protocol describes the manual procedures for purifying RNA using PAXgene Blood RNA tubes and PAXgene Blood RNA Kit based on the manufacturer's recommendation.

3 LIMITATIONS

This protocol is not for the purification of genomic DNA or viral nucleic acid. This protocol has not been validated for all transcripts (only FOS and IL1B gene transcripts); therefore, you should review the manufacturer's data to determine if validation is required.

4 RESPONSIBILITES

Laboratory Personnel	**Responsibility/Role**
PI/Scientific director	1. Determine if the procedures outlined in this SOP for isolation of RNA using PAXgene are appropriate for their study in consideration of sample collection supplies, downstream assays and logistics. 2. Inform Study Investigator if deviations to the protocol are required.
Study Coordinator	1. Ensures that laboratory personnel have been trained in accordance with this SOP before isolating RNA using PAXgene to ensure that the training is documented. 2. Modify the current document or draft a new protocol when deviations are required and ensure all staff are informed and review the revised or new SOP.
Laboratory Technician/Trainee	1. Ensures he/she has read, understands and follows the SOP while isolating RNA using PAXgene RNA isolation Kit 2. Conducts and assists with RNA isolation procedures. 3. Records and documents outcomes. 4. Record and notify the Study coordinator of any deviations from this procedure, which are not accounted for in study specific procedures.

5 SOP SPECIFIC NEEDS AND REQUIREMENTS

5.1 Personnel requirements

5.1.1 All personnel that are involved in sample processing for DNA must be familiar with guidelines for handling RNA to prevent contamination (package insert page 47).

5.1.2 All personnel that work in the lab must be trained in procedures involved in handling biological specimen

5.2 Sample requirements

5.2.1 Samples collected in PAXgene Blood RNA Tubes must be processed within 3 days if stored at ambient temperature (18–25°C) or within 5 days if refrigerated (2–8°C).

5.3 Supplies/Equipment required but not supplied

5.3.1 Lab coat

5.3.2 Hand gloves

5.3.3 PAXgene Blood RNA Kit

5.3.4 PAXgene Blood RNA tube containing sample

5.3.5 Molecular grade absolute Ethanol **(96-100%)**

5.3.6 Nulease free sterile Sterile, aerosol barrier, RNAse-free pipette tip

5.3.7 Graduated cylinders

5.3.8 Centrifuge (3000-5000 xg) with swing out rotor Centrifuge

5.3.9 Vortex mixer

5.3.10 Centrifuge buckets/adapters for PAXgene tube

5.3.11 Crushed ice

5.3.12 Permanent marker for labeling/cryomarker

5.3.13 Micro centrifuge 1000-8000 xg

5.3.14 Shaker-incubator (55°C and 65°C and shaking at 400 rpm, not exceeding 1400 rpm)

5.4 Kit Contents: Store 2–8°C immediately upon receipt

5.4.1 Buffer BR1 (re-suspension): 20 ml

5.4.2 Buffer BR2 (Binding Buffer): 18 ml

5.4.3 Buffer BR3 (Wash Buffer): 45 ml

5.4.4 Buffer BR4 (Wash Buffer): 11 ml

5.4.5 Buffer BR5 (Elution Buffer): 6 ml

5.4.6 RNase-Free Water (bottle): 2 x 125 ml

5.4.7 Proteinase K (green lid): 2 x 1.4 ml

5.4.8 PAXgene RNA Spin Columns (red): 5 x 10

5.4.9 Processing Tubes (2 ml): 6 x 50

5.4.10 Secondary BD Hemogard Closures: 50

5.4.11 Micro centrifuge Tubes (1.5 ml): 3 x 50, 1 x 10

5.4.12 DNase I, RNase-Free (lyophilized): 1500 Kunitz units

5.4.13 Buffer RDD (white lid): 2 x 2 ml

5.4.14 RNase-Free Water (tube, lilac lid): 2 ml

5.4.15 PAXgene Shredder Spin Columns (lilac): 5 x 10

5.4.16 PAXgene Blood RNA Kit Handbook (Version 2): 1

6 ABBREVIATIONS/DEFINITIONS

6.1.1 DNA – deoxyribonucleic acid

6.1.2 µg – microgram

6.1.3 µl – microliter

6.1.4 ml – milliliter

6.1.5 RNA – ribonucleic acid

7 SPECIAL SAFETY PRECAUTIONS

7.1 Personal Protective Equipment (PPE) must be worn while working with biological and chemical materials to prevent personal injuries.

7.2 Buffers BR2 and BR3 contain guanidine thiocyanate, which can form highly reactive compounds when combined with bleach. Therefore, do not clean sample-preparation waste, BR2 or BR3 spills directly with bleach, use detergent and water. If the spill contains potentially infectious agents clean with 1% sodium hypochlorite after detergent and water.

7.3 The RNA stabilizing solution and blood mixture from the PAXgene Blood RNA Tube can be disinfected using 1 volume of commercial bleach solution (5%

sodium hypochlorite) per 9 volumes of the RNA stabilizing solution and blood mixture.

7.4 Please refer to page 7 of the package insert or MSD ((http://www.qiagen.com/Knowledge-and-Support/Troubleshooting-and-Support/QA-QC-Safety-Data/Safety-DataSheets/?catno=000000000000762174) for details regarding chemical hazards of buffer BR2, BR3, Proteinase K and DNase I.

8 MAINTENANCE & CALIBRATION

8.1 Equipment should be maintained and calibrated in accordance with the Manufacturers Manual and Equipment SOP specifications.

8.2 Reference can be made to the equipment SOPs using the SOP Master File using the name of the equipment and document control number(s).

9 QUALITY CONTROL

9.1 All purified RNA samples should be subjected to Quality Control criteria specified in the RNA Quality Control SOP and in accordance to RNA QC procedures established by the facility.

10 PROCEDURE

Manual and automated procedures consist of: centrifugation to pellet nucleic acid, washing, re-suspension and purification.

Manual PAXgene Blood RNA Procedure

10.1.1 Bring DNase I, Buffer RDD, RNase-free water (tube), and refrigerated/frozen samples to room temperature (15–25°C). Samples should equilibrate for at least 2 hours to achieve cell lysis. (Incubation at room temperature overnight may increase RNA yield).

10.1.2 Check buffer BR2 for precipitates. If precipitates have formed, warm the buffer at 37°C to dissolve.

10.1.3 If using RNase-Free DNase set for the first time, dissolve the DNase 1 solid (1500 Kunitz units) in 550µl of the RNase-Free water (provided) to form the DNase stock solution. If the DNase stock solution will be used within 6 weeks, store it at 2-8°C. If the DNase stock solution will not be used within 6 weeks prepare single-use aliquots using the 1.5ml tubes provided. Do not refreeze after thawing or use after 9 months.

Note: DNase 1 is especially sensitive to physical denaturation. Mixing should only be carried out by gently flicking the tube. Do not Vortex.

10.1.4 Add 4 volumes of 96-100% ethanol to the buffer BR4 concentrate to form the buffer BR4 working solution. The instructions are also indicated on the bottle.

10.1.5 Centrifuge the PAXgene Blood RNA Tube(s) at 3000-5000 x g for 10 minutes using a swing-out rotor.

10.1.6 Remove the supernatant by decanting or pipetting. Make sure not to wet the rim, or dry with clean paper towel.

Note: incomplete removal of supernatant will inhibit lysis and dilute the lysate, and therefore inhibit the condition for binding the RNA to the PAXgene membrane.

10.1.7 Add 4ml RNase-free water to the pellet, and close the tube using fresh secondary BD Hemogard closure (supplied with kit). Vortex until the pellet is visibly dissolved.

Note: Small debris in the supernatant prior to the next step (centrifugation) will not affect the procedure.

10.1.8 Centrifuge for 10 minutes at 3000 – 5000 x g using a swing-out rotor. While the samples are spinning, label one 1.5ml micro centrifuge tube per sample.

10.1.9 Remove and discard supernatant.

10.1.10 Note: Any supernatant remaining in the tube will inhibit lysis and dilute the lysate; thereby, affecting binding of RNA to the Paxgene membrane. Add 350µl buffer BR1, and vortex till the pellet is visibly dissolved.

10.1.11 Transfer the sample to a labeled 1.5ml micro centrifuge tube.

10.1.12 Add 300µl buffer BR2 and then add 40µl proteinase K (Do Not mix buffer BR2 and proteinase K together before adding them to the sample.). Mix by vortexing for 5 seconds, and incubate for 10 minutes at 55°C using a shaker-incubator at 400-1400 rpm. During the incubation label one PAXgene Shredder spin column (lilac) per sample and place it in a 2ml processing tube. After the incubation, set the temperature of the shaker-incubator to 65°C.

10.1.13 Pipette the lysate directly into the corresponding labeled PAXgene Shredder spin column and centrifuge for 3 minutes at a maximum speed (Do Not exceed 20,000 x g). During centrifugation label a fresh 1.5ml micro centrifuge tube per sample.

10.1.14 Carefully transfer all supernatant of the flow-through fraction to the labeled 1.5ml tube without disturbing the pellet in the processing tube.

10.1.15 Pipette 350µl 96-100% ethanol. Mix by vortexing, and quick spin (centrifuge 1-2 seconds at 500-1000 x g) to remove drops from the inside of the tube lid

10.1.16 Label a PAXgene RNA spin column (red) for each sample and place it inside a new 2ml processing tube.

10.1.17 Pipette 700µl sample into the PAXgene RNA spin column, and centrifuge for 1 minute at 8000-20,000 x g.

10.1.18 Transfer the PAXgene RNA spin column to a new 2ml processing tube and discard the old processing tube containing flow-through.

10.1.19 Pipette the remaining sample into the PAXgene RNA spin column, and centrifuge for 1 minute at 8000-20000 x g.

10.1.20 Transfer the spin column to a new 2ml processing tube, and discard the old processing tube containing flow-through.

10.1.21 Pipette 350µl buffer BR3 into PAXgene RNA spin column, and centrifuge for 1minute at 8000-20,000 x g.

10.1.22 Transfer the spin column to a new 2 ml processing tube, and discard the old processing tube containing flow-through.

10.1.23 Label a 1.5ml micro centrifuge tube DNase I mix. Multiply the number of samples x 10 to determine the amount of previously prepared DNase 1 stock solution to add to the tube and add the calculated amount to the tube. (Ex. 10ul for sample and 100ul for 10 samples)

10.1.24 Multiply the number of samples by 70 to determine the amount of Buffer RDD to add to the DNase I mix and add the calculated amount to the tube. (Ex. 70µL for 1 sample and 700ul for 10 samples)

10.1.25 Mix the DNase I mix by gently flicking the tube. (Do not vortex because it can denature the DNase 1). Centrifuge the tube for 1-2 seconds to collect residual liquid from the sides of the tube. During centrifugation set the timer to 15 minutes.

10.1.26 Pipette 80ul of DNase 1 mix directly onto the PAXGene RNA spin column membrane. (If part of it is applied to the walls or O ring of the column, rather than the membrane, the DNase digestion will be incomplete). Start the timer and incubate at room temperature (20-30°C) for 15 minutes.

10.1.27 Pipette 350µl buffer BR3 into the PAXGene RNA spin column, and centrifuge for 1 minute at 8000-20,000 x g. Transfer the spin column to a new 2ml processing tube, and discard the old processing tube containing flow-through.

10.1.28 Pipette 500µl buffer BR4 (previously prepared) to the PAXGene RNA spin column, and centrifuge for 1 minute at 8000-20000 x g. Transfer the spin column to a new 2ml processing tube, and discard the old processing tube containing flow-through.

10.1.29 Pipette another 500µl buffer BR4 to the PAXgene RNA spin column. Centrifuge for 3 minutes at 8000-20000 x g.

10.1.30 Discard the processing tube containing flow-through, and transfer the PAXgene RNA spin column to a new 2ml processing tube. Centrifuge for 1 minute at 8000-20000 x g. During centrifugation label a 1.5ml micro centrifuge tube for each sample.

10.1.31 Discard the tube containing the flow-through. Place the PAXgene RNA spin column into the corresponding labeled 1.5ml micro centrifuge tube.

10.1.32 Pipette 40µl buffer BR5 directly onto the PAXgene RNA spin column membrane, ensuring the entire membrane is wet with buffer BR5 for maximum elution efficiency. Centrifuge for 1 minute at 8000-20000 x g to eluate the RNA.

10.1.33 Repeat the elution step as described in step 10.1.32, using 40µl buffer BR5 and the same micro centrifuge tube.

10.1.34 Incubate the elute for 5 minute at 65°C in the shaker-incubator without shaking. It is important that the incubation be monitored by timer to prevent exceeding the 5 minutes specified as increased time and temperature may affect downstream applications.

10.1.35 Chill immediately on ice

10.1.36 Refer to the RNA Quality Control SOP for detailed RNA QC guidelines. For quantification in Tris buffer, use the relationship $A_{260}=1 \rightarrow 44$ µg/ml.

10.1.37 If the RNA samples will not be used immediately, store at -70°C. However, if a -70C freezer is unavailable, store the samples at-20C (not recommended for long term storage).

11 REVISION HISTORY

Revision No	Effective Date	Description of Changes Made from Preceding Revision	Approved by/ Date

ANNEX 1: DOCUMENTATION OF SUGGESTED CHANGES TO THIS SOP

CLAUSE	SUGGESTION	BY	DATE