

## Protocol Name: RNA Pax v1

### Notes:

- draw 2.5ml of blood directly into PAXgene tube and invert the tube 10 times immediately, do not shake
  - before starting RNA purification procedure, ensure that the tubes have been incubated at room temperature for at least 2 hours in order to ensure complete cell lysis. this incubation can be carried out either before or after storage at  $-20^{\circ}\text{C}$  or below.
  - if tubes were immediately frozen after blood collection, then after removal from storage, first equilibrate to room temperature for at least 2 hours and then incubate at room temperature for an additional 2 hours. after thawing, invert the tubes 10 times.
  - set shaker-incubator to  $55^{\circ}\text{C}$
  - set BioOven to  $65^{\circ}\text{C}$
  - thaw Dnase I stock solution for on-column Dnase digestion
1. Centrifuge the PAXgene Blood RNA Tube for 10 minutes at  $3345\text{g}/4000\text{rpms}$ , room temperature, brake on using a swing-out rotor with adapters for round-bottom tubes.
  2. Remove the supernatant by decanting into 15ml conical tube and discard. Dry the rim of the tube with a Kim-wipe. Add 5ml Rnase free water to the pellet, and close the tube using a fresh secondary Hemogard closure.
  3. Thoroughly resuspend the pellet by pulse vortexing with BioAnalyzer vortexer set at  $\sim 2200$ . Centrifuge for 10 minutes at  $3345\text{g}/4000\text{rpms}$ , room temperature, brake on. Remove the entire supernatant by decanting and discard.
  4. Add 360ul Buffer BR1 and vortex until the pellet is visibly dissolved.
  5. Pipet the sample into a 1.5ml microcentrifuge tube. Add 300ul Buffer BR2 and 40ul Proteinase K. Mix by vortexing, and incubate for 10 minutes at  $55^{\circ}\text{C}$  using a shaker-incubator at 1400rpm (setting 4.0) with tubes taped to surface.
  6. Centrifuge the sample for 3 minutes at maximum speed in a microcentrifuge, and transfer the supernatant to a fresh 1.5ml microcentrifuge tube.
  7. Add 350ul 100% ETOH. Mix by vortexing, and quick spin for 1-2 seconds to remove drops from inside of the tube lid.
  8. Add 700ul sample to a PAXgene spin column place in a 2ml processing tube, and centrifuge for 1 minute at maximum speed. Place the PAXgene spin column in a new 2ml processing tube, and discard the old processing tube containing the flow-through
  9. Add remaining sample to the PAXgene spin column, and centrifuge for 1 minute at maximum speed. Place the PAXgene spin column in a new 2ml processing tube, and discard the old processing tube containing the flow-through.
  10. Add 350ul Buffer BR3 to the PAXgene spin column. Centrifuge for 1 minute at maximum speed. Transfer the PAXgene spin column to a new processing tube and discard the old processing tube containing the flow-through.
  11. Add 10ul Dnase I stock solution to 70ul Buffer RDD. Mix by gently flicking the tube, and centrifuge briefly to collect residual liquid from the sides of the tube.

12. Add the Dnase I incubation mix (80ul) directly onto the PAXgene spin column membrane, and incubate on the bench top for 15 minutes.
13. Add 350ul Buffer BR3 to the PAXgene spin column. Centrifuge for 1 minute at maximum speed. Place the PAXgene spin column in a new 2ml processing tube, and discard the old processing tube containing the flow-through.
14. Add 500ul Buffer BR4 to the PAXgene spin column, and centrifuge for 1 minute at maximum speed. Place the PAXgene spin column in a new 2ml processing tube, and discard the old processing tube containing the flow-through.
15. Add another 500ul Buffer BR4 to the PAXgene spin column. Centrifuge for 3 minutes at maximum speed to dry the PAXgene spin column membrane. Place the PAXgene spin column in a new 2ml processing tube, and discard the old processing tube containing the flow-through. Centrifuge for 1 minute at maximum speed.
16. Place the PAXgene spin column in a 1.5ml elution tube and discard the old processing tube containing the flow-through. Add 40ul Buffer BR5 directly onto the PAXgene spin column membrane. Centrifuge for 1 minute at maximum speed to elute the RNA. Do not discard the eluate.
17. Add another 40ul Buffer BR5 directly onto the PAXgene spin column membrane. Centrifuge for 1 minute at maximum speed to elute further RNA. Do not discard the eluate.
18. Incubate the eluate for 5 minutes at 65°C in BioOven. After incubation, chill immediately on ice.
19. Quantify RNA using NanoDrop.