# GeneLab Standard Operating Procedure: RNA isolation from blood using RiboPure RNA Purification kit

*June 2022*

*Version 1.0*

# Document Revisions

| Document Number | Revision Number | Date | Description of Changes |
|---|---|---|---|
| GL-SOP-3.5 | 1.0 | June 2022 | Original document |
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# Scope and Purpose

This procedure describes the steps required to extract RNA from mammalian whole blood samples of 300-500uL, in RNAlater or freshly collected. The extraction kit used in this procedure is RiboPure Blood Kit (Thermo Fisher Scientific). In addition, in this procedure we describe steps for depleting the isolated RNA from residual DNA using DNase I. This step is required for RNA that will be used for sequencing. It is strongly advised to read the RiboPure Blood Kit Protocol in full before starting this procedure.

At this time, we validated this procedure using rat and mouse blood collected in RNAlater and stored at -80°C, for procedure adjustments for other collection and storage methods – consult the kit protocol.

# Equipment and Consumables

1. Eppendorf Centrifuge 5424/5424 R
2. Bench top microcentrifuge to accommodate 1.5mL tubes (Thermo Scientific Cat #75004081 or similar)
3. Heat block (Eppendorf Thermomixer C Cat #2231000667 or similar)
4. DNA LoBind Microcentrifuge Tubes1.5mL (Thermo Scientific, Cat #13-698-791)
5. 2ml polypropylene tubes (Thermo Fisher Scientific, Cat#AM12425 or similar)
6. 5ml polypropylene tubes (Eppendorf, Cat# 0030119401 or similar)

# Reagents

1. RiboPure Blook Kit (Thermo Fisher Scientific, Cat #AM1928)
    a. Elution Solution
    b. Lysis Solution
    c. Acid-Phenol:Chloroform
    d. Sodium Acetate Solution
    e. Wash solution 1
    f. Wash 2/3 Concentrate

g. Filter cartridges
h. Collection tubes
i. DNase I, 8units/uL
j. 20X DNase buffer

2. Non-denatured, mol. grade ethanol, 200 proof (Fisher Scientific, Cat #BP2818100)
3. RNase/DNase free water (Thermo Fisher Scientific, Cat #10977015 or similar)
4. RNaseZap Decontamination Solution (Thermo Fisher Scientific, Cat #AM9782 or similar)

# General Practices and Notes

1. While working with blood samples, make sure to wear all required PPE (gloves, lab coat, goggles, closed toe shoes).
2. Make sure all the required safety training has been completed. (Blood borne pathogens, sharps).
3. Turn the heat block on and set it to 75°C, it will be used during elution.
4. **Make sure 56mL of 100% EtOH is added to 2/3 wash solution prior to first use.**
5. Set a microcentrifuge to room temperature (25°C).
6. Decontaminate all surfaces once the work is completed.

# Procedure

1. Thaw the blood samples on ice until completely liquid and no ice crystals are present.
   For blood in RNAlater, expect to have 1.6-2.0ml of sample per each tube.
2. Briefly vortex the samples and then centrifuge for 5min at maximum speed in room temperature centrifuge.
   a. Blood cells will form a large brown or red-brown pellet. Supernatant will vary in color.
3. Remove and discard the supernatant. Thoroughly remove all liquid – including the portion directly above the cell pellet. Remove liquid from inside the cap.
4. Assess the pelleted cell volume.
   a. If pellet volume is below 0.6ml proceed working in the 2mL cryotube in the next steps.
   b. If pellet volume is above 0.6ml, transfer to 5mL tube in step 5.
5. If 4a – Add 800uL Lysis solution and 50uL of Sodium Acetate into the cryotube with the cell pellet. Vortex rigorously to lyse the blood cells. Check if the solution is homogeneous by inverting the tube. RNAlater preserved blood will require very rigorous homogenization.
   If 4b - Add 800uL Lysis solution and 50uL of Sodium Acetate into a fresh 5mL tube and use the made solution to mix with the cell pellet and transfer it to the new tube. Vortex rigorously to lyse the blood cells. Check if the solution is homogeneous by inverting the tube. RNAlater preserved blood will require very rigorous homogenization.
6. To add Acid-Phenol:Chloroform, set the bottle on the bench and assess the location of the aqueous buffer that covers the chemical. Withdraw 500uL of Acid-Phenol:Chloroform from

below the aqueous layer and add it to the cell lysate. Shake the tubes rigorously or vortex for 30 seconds.

7. Incubate at room temperature for 5 minutes.
8. Centrifuge the tubes for 3min at full speed in a room temperature centrifuge.
   Phase separation should take place; organic (bottom, heme and proteins) and aqueous phases (top, RNA) should be present. The aqueous phase can be turbid/dark in color. Avoid touching the organic phase.
9. Transfer the top aqueous phase containing the RNA into new 2mL tube. Expect 1.0-1.5mL.
10. To each tube of recovered RNA, add half volume of 100% Ethanol. (If recovered 1.2mL of RNA, add 600uL of 100% ethanol).
11. Vortex for 10 seconds.
12. Assemble a filter cartridge for each sample by putting it in to a collection tube and labeling it.
13. Apply 700uL of sample onto the filter cartridge assembly and centrifuge for 30 seconds to pass the liquid through the filter.
14. Discard the flow-through. Place the filter cartridge back onto the collection tube.
15. Load the next 700uL of sample and centrifuge for 30 seconds to pass the liquid through the filter.
16. Discard the flow-through. Place the filter cartridge back onto the collection tube and repeat until all sample passed the filter cartridge.

    - Get the DNase box out of -20C to thaw reagents at RT.
    - Warm up elution solution to 75°C

17. Apply 700uL Wash Solution 1 to the filter cartridge. Centrifuge for 30 seconds to pass the liquid through the filter.
18. Discard the flow-through. Place the filter cartridge back onto the collection tube.
19. Apply 700uL Wash Solution 2/3 to the filter cartridge. Centrifuge for 30 seconds to pass the liquid through the filter.
20. Discard the flow-through. Place the filter cartridge back onto the collection tube.
21. Repeat the 700uL wash with the Wash solution 2/3.
22. Discard the flow-through. Place the filter cartridge back onto the collection tube.
23. Centrifuge the empty filter cartridge in the collection tube for 1min at full speed.
24. Transfer the filter into a new collection tube.
25. Apply 50uL of elution solution at 75°C to the center of the filter. Close cap and incubate at room temperature for 1 minute. Centrifuge for 30 seconds at full speed.
26. Repeat elution with another 50uL of elution solution. Close cap and centrifuge at RT for 1 minute.
27. Set the thermomixer to 37°C

**DNase Treatment**

28. Add 1/20$^{th}$ volume of 20X DNase Buffer and 1uL of DNase I (8U/uL) to the eluted RNA. Mix gently. If RNA was eluted in 100uL, add 4uL of 20X DNase Buffer and 1uL DNase I.
29. Incubate 30 minutes at 37°C
30. Use a volume of DNase Inactivation Reagent equal to 20% of the volume of RNA treated. If 100uL of RNA is treated, add 20uL of DNase inactivation Reagent.
31. Vortex the tube to mix the inactivation reagent with the RNA.
32. Incubate at room temperature for 1 minute.
33. Vortex the tubes.
34. Incubate at room temperature for 1 minute.
35. Centrifuge the sample for 1 minute at full speed to pellet the DNase Inactivation Reagent.
36. Transfer the aqueous RNA solution to a new RNase-Free, Lo-bind tube.
37. Following SOP# 4.1 to perform DNA quantification using Qubit fluorimeter.
38. Following SOP# 4.2 to perform DNA quality analysis using TapeStation.
39. Aliquot the generated samples following SOP#1.1.