RNA Easy/Isopure™ for Paxgene Blood RNA

Catalog Numbers: R9210-S, R9210-M, R9210-L

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INTRODUCTION

The Aline RNA Easy™ kit for Paxgene blood utilizes Aline's unique paramagnetic bead technology to isolate cell free DNA and RNA from plasma, serum or RNA from blood. This protocol provides instructions to extract cRNA from 400uL blood sample in a 2.2 mL 96-well format. 5mL of plasma may be extracted with a larger tube. Purification begins by adding a lysis buffer to release DNA/RNA. DNA/RNA is immobilized on magnetic particles. Contaminants can then be washed away with simple washing procedure. DNA is further removed by a Dnase I digestion step, leaving the RNA ready for elution from the magnetic particles. The 96-well plate format procedure is highly amenable to automation due to the utilization of magnetic separation. Vacuum filtration or centrifugation procedure is eliminated. RNA can be efficiently recovered.

The major advantage of the kit is that large number of blood samples can be processed with automation. Each reaction in the kit is enough for processing 400uL blood samples. There is no heme carryover in eluate.

RNA prepared from the Aline RNA Easy™ Kit can be used in the following applications:

- Next gen library construction.
- PCR amplification
- Human identity testing
- Microarray hybridizations

Process Overview:

1. Lyse samples at 55°C with proteinase K.
2. Addition of Magnetic Beads/Isopropanol Buffer.
3. Magnetic beads separation from supernatant.
4. Wash the magnetic beads with wash buffer and ethanol.
5. DNase I digestion for RNA only recovery..
6. Rebinding with Re-Bind2 Buffer.
4. Magnetic beads separation from supernatant.
5. Elution.

Reagents supplied in the kit:

The reagents have a shelf life of 12 months if stored as directed.

Reagent Description Storage Condition Upon Arrival

Lysis Buffer: Room Temperature.
Magnetic Beads (MB): Store at 2-8°C.
Wash Buffer (WB): Store at Room Temperature.
Proteinase K Store at -20°C.
Re-Bind2 Buffer Store at 2-8°C.

Materials to be supplied by users:
96-well plates: 2.2mL and 1.2 mL (for plate format) or **2 mL tube** (for tube format)
Ethanol: 70-80%
Isopropanol: 100%
RNase Free water for RNA elution.
DNase I (RNase -free) and buffer (2U/uL); Ambion #AM2224
55°C and 37°C Water Baths.
Liquid handling robotics or a multi-channel hand pipette.

TOTAL RNA ISOLATION FROM PAXGENE PRESERVED BLOOD PROCEDURE

Aline Biosciences strongly recommends using aerosol-barrier (filter) pipette tips whenever necessary.

Part A — Reagent Preparation

Prepare the following reagents in advance for both the 96-well and 2mL tube protocols:
1. If a separate PK buffer is supplied, add the Proteinase K Buffer to the Proteinase K tube/bottle as specified on the bottle. Mix by inverting the tube/bottle several times. Do not vortex to avoid foaming. Solution will initially appear cloudy after mixing. Let the solution sit for about 5 minutes to clear prior to using. Keep the Proteinase K solution at -20°C until use.

2. Add 100% isopropanol to Wash Buffer bottle as specified on the bottle. This only needs to be done once.

3. Prepare **Magnetic Beads/Isopropanol** Solution:

Completely re-suspend the beads by shaking the Magnetic Bead bottle. Add 100% isopropanol to magnetic beads bottle as specified on the bottle. This only needs to be done once.

4. Prepare DNase Solution if DNA is to be removed:
100 µL of 1X DNase solution are required per reaction. Combine 85 µL nuclease-free water, 10 µL 10X DNase buffer, and 5 µL of DNase I. Make this solution fresh for each set of samples.

Part B — 2.2 mL 96 well Plate Protocol

Thaw frozen tubes of PAXgene blood at room temperature. Cap the tubes tightly then mix by inverting each tube several times or by vortexing. Bring the tube temperature to room temperature.

1. Aliquot 400 µL of PAXgene preserved blood into each well of a 2.2 mL processing plate.

2. **Add Proteinase K and Lysis Buffer:**
- Add 20 µL of Proteinase K (50mg/mL, prepared in Part A step 1)
- Add 300 µL of Lysis Buffer
Mix thoroughly by pipetting up and down 10 times.

3. **Lysis and Protein Digestion:**
Seal plate with a plate seal. Incubate samples in water bath at 55°C for 15 minutes. Before proceeding to the next step, let the samples cool for 2 minutes to room temperature.

NOTE*: When using this plate in conjunction with a water bath, make sure the plate does not tip over and the seal does not get wet. Should the seal get wet or condensation form on the seal, spin the liquid down and very carefully remove the seal.*

4. **Add 410 µL Magnetic Beads/Isopropanol Solution (prepared in Part A Step 3) to the samples and pipette mix 10 times. Incubate samples at room temperature for 5 minutes.**

NOTE: Shake or tipmix Magnetic Beads/Isopropanol Solution to disperse beads before adding to sample.

5. P**lace 2.2 mL processing plate on Agencourt SPRIPlate 96R Super Magnet Plate and separate for 10 minutes.**

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6. **Fully remove supernatant from the 2.2 mL processing plate and discard.**

NOTE: The following technique is recommended when working with opaque supernatant: Place the pipette tip on the side of the well and carefully aspirate the liquid by following the liquid level down until approximately 200-250 µL remains in the well. Next, carefully place liquid level down until approximately 200-250 µL remains in the well. Next, carefully place the pipette tip at the center of the bottom of the well and slowly aspirate the remaining liquid, revealing the ring of beads.

7. **Remove the 2.2 mL processing plate from the magnet and wash the beads by adding 800 µL of Wash Buffer. (Isopropanol must be added to Wash Buffer before using the kit for the first time – See Part A step 2). Pipette mix 10 times to resuspend the magnetic beads.**

8. **Transfer the suspension to a 1.2 mL processing plate. Be sure to transfer all of the sample and magnetic beads to the new plate.**

NOTE: Transferring the samples to the smaller plate allows for easier pipetting in subsequent steps.

9. **Place 1.2 mL processing plate on the magnet and separate for 7 minutes. Wait for the solution to clear before proceeding to the next step.**

10. **Completely remove supernatant from the 1.2 mL processing plate and discard. NOTE:This step must be performed while the plate is situated on the magnet. Do not to disturb the ring of separated magnetic beads.**

11. **Remove the 1.2 mL processing plate from the magnet and add 800 µL 70% ethanol. Pipette mix 5 times to resuspend the magnetic beads.**

12. R**Return 1.2 mL processing plate to the magnet for 3 minutes.**

13. **Remove as much ethanol as possible and allow magnetic beads to dry for 5 minutes at room temperature.**

NOTE: Pipette slowly to avoid disturbing the beads. If too much ethanol is present (more than 5 µL),

14. **Remove the 1.2 mL processing plate from the magnet and add 100 µL of DNase solution (prepared in Part A Step 4). Pipette mix 5 times carefully - avoid bubbles and foaming.**

15. **Seal plate with a plate seal and incubate 1.2 mL processing plate in water bath for 15 minutes at 37ºC.**

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16. ***DO NOT REMOVE THE DNase SOLUTION*. Add 200 µL of Re-Bind 2 Buffer and pipette mix 10 times. Incubate at room temperature for 5 minutes.**

17. Place 1.2 mL processing plate onto the magnet for 5 minutes. Wait for the solution to clear before proceeding to the next step.

18. Remove supernatant and discard. Wash by adding 800 µL of 70% ethanol. Do Not Place 1.2 mL processing plate onto the magnet for 5 minutes. Wait for the solution to clear before proceeding to the next step. Pipette Mix. Let sit for approximately one minute and then remove ethanol while processing plate remains situated on the magnet plate.

19. Repeat step #18 one more time for a total of 2 ethanol washes.

20. Allow magnetic beads to dry for 10 minutes at room temperature.
Repeat step #18 one more time for a total of 2 ethanol washes. Beads do not need to be completely dry, but the traces of liquid should be gone (i.e. droplets or puddles).

21. Remove 1.2 mL processing plate from the magnet and elute RNA by adding a minimum of 20 µL of nuclease free water. Pipette mix 10 times and incubate at room temperature for 2 minutes.
On average, a 20 µL elution will produce a 20-50 ng/µL solution of RNA.

22. Return the plate to the magnet for 2 minutes and carefully transfer eluted RNA away from the beads and into a fresh plate for storage.

Part C —2 mL Tube Protocol

Thaw frozen tubes of PAXgene blood at room temperature. Cap the tubes tightly then mix by inverting each tube several times or by vortexing. Bring the tube temperature to room temperature.

1. Aliquot 400 µL PAXgene preserved blood into a 2 mL microcentrifuge tube.

2. **Add Proteinase K and Lysis Buffer:**
- Add 20 µL of Proteinase K (50mg/mL, prepared in Part A step 1)
- Add 300 µL of Lysis Buffer
Mix thoroughly by pipetting up and down 10 times.

3. **Lysis and Protein Digestion:**

Cap the tube. Incubate samples in water bath at 55°C for 15 minutes. Before proceeding to the next step, let the samples cool for 2 minutes to room temperature.

4. **Add 410 µL Magnetic Beads/Isopropanol Solution (prepared in Part A Step 3) to the samples and mix by vortexing the tube. Incubate samples at room temperature for 5 minutes.**

NOTE: Shake or tipmix Magnetic Beads/Isopropanol solution to disperse beads before adding to sample.

5. **Place tubes on an Agencourt SPRIstand (6 Position Tube Magnet) and separate for 10 minutes.**

6. **Fully remove supernatant from tube and discard.** This step must be performed while the tube is situated on the magnet stand.

NOTE: The following technique is recommended when working with opaque supernatant: Place the pipette tip on the side of the tube opposite the magnet and carefully aspirate the liquid while following
the liquid level down.

7. **REMOVE tube from the magnet stand and wash the beads by adding 800 µL Wash Buffer. (Isopropanol must be added to Wash Buffer before using the kit for the first time – See Part A step 2.)**
Pipette mix 10 times to resuspend the magnetic beads.

8. **Transfer the suspension to a fresh 2 mL tube. Be sure to transfer all of the sample and magnetic beads to the new tube.**

NOTE: Transferring is important so later steps of the protocol won't be contaminated with blood solution that may have collected on the lid of the first tube.

10. Completely remove supernatant from the tube and discard.

NOTE: This step must be performed while the tube is situated on the magnet stand. Place the pipette tip at the bottom of the tube and carefully aspirate the liquid so the layer of separated magnetic beads on the side of the tube is not disturbed.

11. Leave tube on the magnet stand and add 800 µL 70% ethanol. Let sit for approximately one minute and then remove ethanol while the tube is on the magnet stand.

12. Remove as much ethanol as possible from the bottom of the tube and dry for 5 minutes at room temperature.

NOTE: Pipette slowly to avoid disturbing the beads. If too much ethanol is present (more than 5 µL), the DNase digestion will be inhibited thereby affecting downstream applications.

13. Remove the tube from the magnet and add 100 µL of DNase solution (prepared in Part A Step 4). Pipette mix 5 times carefully - avoid bubbles and foaming.

14. Cap the tube. Incubate the samples in water bath at 37ºC for 15 minutes.

15. *DO NOT REMOVE THE DNase SOLUTION*. Add 200 µL of Bind 2 Buffer and pipette mix 10 times. Incubate at room temperature for 5 minutes.

16. Place the tube on the magnet stand for 5 minutes. Wait for the solution to clear before proceeding to the next step.

17. Remove supernatant and discard. Wash by adding 800 µL of 70% ethanol. Do Not Pipette Mix. Let sit for approximately one minute and then remove ethanol while tube remains situated on the magnet stand.

18. Repeat step #16 one more time for a total of 2 ethanol washes.

19. Allow magnetic beads to dry for 10 minutes at room temperature.

NOTE: Beads do not need to be completely dry, but the traces of liquid should be gone (i.e. droplets or puddles).

20. Remove the tube from the magnet stand and elute RNA by dissolving the beads pellet in a minimum of 20 µL of nuclease free water. Pipette mix 10 times and incubate at room temperature for 2 minutes.
On average, a 20 µL elution will produce a 20-50 ng/µL solution of RNA.

21. Return the tube to the magnet stand for 2 minutes and carefully transfer eluted RNA away from the beads and into a fresh tube for storage.

PAXGEN BLOOD DNA/RNA ISOLATION PROCEDURE

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If you have any technical questions, please feel free to contact support@Alinebiosciences.com or 1-888-987-3677.

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