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Working

Collection and RNA Isolation from stabilized Whole Blood using Tempus™ Blood RNA Tube and Spin RNA Isolation Reagent Kit

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ABSTRACT

This protocol was used to isolate RNA from whole blood samples of humans for quantitative real time PCR.

MATERIALS

NAME	CATALOG #	VENDOR
TempusTM Blood RNA Tube	4342792	Thermo Fisher Scientific
Spin RNA Isolation Reagent Kit	4380204	Thermo Fisher Scientific

1 • Draw 3 mL of blood directly into the Tempus Blood RNA Tube, following clinic's standard procedures for drawing blood from individuals into blood collection tubes containing liquid reagents.

2 • Immediately after the Tempus tube is filled, stabilize the blood by shaking the tube vigorously or vortexing the contents for 10 seconds to ensure that the Applied Biosystems Stabilizing Reagent makes uniform contact with the sample.

3 • Store or ship Tempus tubes containing stabilized samples at room temperature within 5 days or less.

4 • Remove the cap from the Tempus tube, then pour the contents of the tube into a clean 50-mL tube.

5 • Pipet 3 mL of 1× PBS (Ca2+/Mg2+-free) into the tube to bring the total volume to 12 mL.

6 • Replace the cap on the tube, then vortex the tube vigorously (at maximum vortex speed) for 30 seconds to ensure proper mixing of the contents.

7 • Centrifuge the tube at 4 °C at 3,000 x g (rcf) for 30 minutes.

8 • Carefully pour off the supernatant.

9 • Leave the tube inverted on absorbent paper for 1 to 2 minutes.

10 • Blot the remaining drops of liquid off the rim of the tube with clean absorbent paper.

11 • Pipet 400 µL of RNA Purification Resuspension Solution into the tube, then vortex briefly to resuspend the RNA pellet.

12 • The resuspended RNA can be kept on ice while preparing for the next steps.

13 • Label the RNA purification filter, then insert the filter into a waste collection tube.

14 • Pre-wet the filtration membrane by pipeting RNA Purification Wash Solution 1 into the purification filter.

15 • Pipet the resuspended RNA into the purification filter, then centrifuge (16,000 x g for 30 sec).

16 • Remove the purification filter, discard the liquid waste collected in the waste tube, then re-insert the purification filter into the waste tube.

17 • Pipet RNA Purification Wash Solution 1 into the purification filter, then centrifuge (16,000 x g for 30 sec).

18 • Remove the purification filter, discard the liquid waste collected in the waste tube, then re-insert the purification filter into the waste tube.

19 • Pipet RNA Purification Wash Solution 2 into the purification filter, then centrifuge (16,000 x g for 30 sec).

20 • Remove the purification filter, discard the liquid waste collected in the waste tube, then re-insert the purification filter into the waste tube.

21 • Pipet RNA Purification Wash Solution 2 into the purification filter, then centrifuge (16,000 x g for 30 sec).

22 • Remove the purification filter, discard the liquid waste collected in the waste tube, then re-insert the purification filter into the waste tube.

23 • Centrifuge (16,000 x g for 30 sec) to dry the membrane.

24 • Transfer the purification filter to a new, labeled collection tube to collect the eluate.

25 • Pipet 50 µL Nucleic Acid Purification Elution Solution into the purification filter, close the cap, incubate the entire tube at 70 °C for 2 min, then centrifuge (16,000 x g for 30 sec).

26 • Pipet the collected RNA eluate back into the purification filter, then centrifuge (16,000 x g for 30 sec). No incubation is necessary.

27 • Discard the purification filter, then transfer approximately 45 µL of the RNA eluate to a new, labeled collection tube.

28 • Replace the cap on the new collection tube, then store the RNA at −20 °C, or −80 °C for long-term storage.