Blood Cells Total RNA Purification Protocol

1	Collect blood cells by centrifugation of 0.5 mL of whole blood at 400 × g for 5 min at 4ºC. Blood cells will generate a pellet of approximately 60-70% of the total sample volume. Remove the clear supernatant (plasma) from the pellet with a pipette.
2	Resuspend the pellet in 600 µL of Lysis Buffer supplemented with β-mercaptoethanol or DTT. Vortex or pipet to mix thoroughly.
3	Add 450 µL of ethanol (96-100%) and mix by pipetting.
4	Transfer up to 700 µL of lysate to (the GeneJET RNA Purification Column) inserted in a collection tube. Centrifuge the column for 1 min at ≥12000 × g. Discard the flow- through and place the purification column back into the collection tube. Repeat this step until all of the lysate has been transferred into the column and centrifuged. Discard the collection tube containing the flow-through solution. Place the (GeneJET RNA Purification Column) into a new (2 mL collection tube).
5	Add 700 µL of Wash Buffer 1 (supplemented with ethanol) to the (GeneJET RNA Purification Column) and centrifuge for 1 min at ≥12000 × g. Discard the flow-through and place the purification column back into the collection tube.
6	Add 600 µL of Wash Buffer 2 (supplemented with ethanol, see p. 3) to (the GeneJET RNA Purification Column) and centrifuge for 1 min at ≥12000 × g. Discard the flow-through and place the purification column back into the collection tube.
7	Add 250 µL of Wash Buffer 2 to (the GeneJET RNA Purification Column) and centrifuge for 2 min at ≥12000 × g. Optional. If residual solution is seen in the purification column, empty the collection tube and re-spin the column for 1 min. at maximum speed. Discard the collection tube containing the flow-through solution and transfer (the GeneJET RNA Purification Column) to a sterile (1.5 mL RNase-free microcentrifuge tube) .
8	Add 50 µL of Water, nuclease-free to the center of (the GeneJET RNA Purification Column membrane). Centrifuge for 1 min at ≥12000 × g to elute RNA**.**
9	Discard the purification column. Use the purified RNA for downstream applications or store RNA at -20°C or -70°C until use.