

AccuPrep® Blood RNA Extraction Kit (K-3144)**I Before You Begin**

- 1) Add 10 μ l β -mercaptoethanol per 1 ml RDD Buffer.
- 2) Prepare additional ethanol (96~100%) and Isopropanol that is not included.
- 3) Completely dissolve one vial of **Proteinase K** in **1,250 μ l** of nuclease-free water. For short term storage, dissolved Proteinase K should be stored at **4°C**. For long term storage, it is recommended to aliquot the enzyme into separate tubes and store at **-20°C**.

II RNA Extraction from Whole Blood

- 1) Add **20 μ l** of **Proteinase K** to a 1.5 ml or 2 ml tube.
- 2) Add **200 μ l** of **whole blood** to the tube containing Proteinase K.
- 3) Add **200 μ l** of **RDD Buffer** to the sample and mix immediately by vortexing.
- 4) Incubate the mixture at room temperature for 30 min.
- 5) Add **300 μ l** of **Isopropanol** and mix well by pipetting.
- 6) Carefully transfer the lysate to a binding column in a 2 ml collection tube.
- 7) Close the lid and centrifuge at **$\geq 14,000$ rpm for 20 sec.**
- 8) Discard the flow-through from the collection tube and transfer the binding column tube to a new 1.5 ml tube.
- 9) Add **200 μ l** of **RV Buffer** onto binding column tube, close the tube, and centrifuge at **14,000 rpm for 20 sec.**
- 10) Add **200 μ l** of **ethanol (96~100%)** to the **flow-through (step 9)** and mix well by pipetting.
- 11) Transfer the mixture to a new binding column in a 2 ml collection tube, close the tube, and centrifuge **14,000 rpm for 20 sec.**
- 12) Discard the solution from the collection tube and reuse the collection tube.
- 13) Add **700 μ l** of **RWA1 Buffer** without wetting the rim, close the tube, and centrifuge at **14,000 rpm for 20 sec.**
- 14) Discard the solution from the collection tube and reuse the collection tube.
- 15) Add **500 μ l** of **RWA2 Buffer** without wetting the rim, close the tube, and centrifuge at **14,000 rpm for 20 sec.**
- 16) Discard the solution from the collection tube and reuse the collection tube.
- 17) Add **500 μ l** of **RWA2 Buffer** without wetting the rim, close the tube, and centrifuge at **14,000 rpm for 2 min.**
- 18) Discard the solution from the collection tube and reuse the collection tube.
- 19) Centrifuge once more at **14,000 rpm for 1 min** to completely remove ethanol, and check that there is no droplet clinging to the bottom of binding column tube.

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- 20) Transfer the binding column tube to a new 1.5 ml tube for elution, add at least **30 µl** of **ER Buffer** onto binding column tube, and wait for at least 1 min at RT (15~25°C).
- 21) Centrifuge at **10,000 rpm** for **1 min** to elute.

※ For more information, please visit www.bioneer.com and refer to the User's Guide of this kit.