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One page protocol

AccuPrep® Blood RNA Extraction Kit (K-3144)

Ⅰ Before You Begin

1) Add 10 µl 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 µl** 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 µl** of **Proteinase K** to a 1.5 ml or 2 ml tube.
2) Add **200 µl** of **whole blood** to the tube containing Proteinase K.
3) Add **200 µl** of **RDD Buffer** to the sample and mix immediately by vortexing.
4) Incubate the mixture at room temperature for 30 min.
5) Add **300 µl** 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 **≥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 µl** of **RV Buffer** onto binding column tube, close the tube, and centrifuge at **14,000 rpm** for **20 sec**.
10) Add **200 µl** 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) Add **700 µl** of **RWA1 Buffer** without wetting the rim, close the tube, and centrifuge at **14,000 rpm** for **20 sec**.
13) Discard the solution from the collection tube and reuse the collection tube.
14) Add **500 µl** of **RWA2 Buffer** without wetting the rim, close the tube, and centrifuge at **14,000 rpm** for **20 sec**.
15) Discard the solution from the collection tube and reuse the collection tube.
16) Add **500 µl** of **RWA2 Buffer** without wetting the rim, close the tube, and centrifuge at **14,000 rpm** for **2 min**.
17) Discard the solution from the collection tube and reuse the collection tube.
18) 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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19) 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℃).
20) 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.

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