RERNAKIA

High Pure Blood RNA extraction kit

Cat. No:

FPKT032.0025

FPKT032.0050

FPKT032.0100

Contents:

Components	25 preps	50 preps	100 preps
RLT1 buffer	50 ml	50 ml*2	50 ml*4
RLT2 buffer	10 ml	20 ml	40 ml
WR1 buffer	10 ml	20 ml	40 ml
WR2 buffer	2.5 ml	5 ml	10 ml
ER buffer	1.5 ml	3 ml	5 ml
DNase-RNase Free (1 mg/ ml)	125 µl	250 µl	500 µl
10x DNase Incubation Buffer	125 µl	250 µl	500 µl
Column and collection tubes	25 each	50 each	100 each

Kit storage:

⚠ This kit should be stored at room temperature.

⚠ **DNase** should be stored at -20 °C

If properly stored, all kit components are stable until the expiration date printed on the label.

Additional Equipment and Reagent required

• Make sure everything is RNase-free when handling RNA.
• Absolute ethanol
• ß-Mercaptoethanol
• Standard tabletop microcentrifuge capable of 13,000 x *g* centrifugal force
• Microcentrifuge tubes, 1.5 ml, sterile
• liquid nitrogen & mortar

Application

The High Pure RNA Isolation Kit is designed to extract RNA from blood samples. The RNA can then be used for downstream applications such as gene expression analysis, qPCR, microarray analysis, and next-generation sequencing.
The general steps involved in using a blood RNA isolation kit include:
1. Collect the blood sample using standard venipuncture techniques and transfer it into a collection tube containing a stabilizing reagent.
2. Centrifuge the collection tube to separate the blood cells from the plasma or serum.
3. Transfer the plasma or serum to a fresh tube and add a lysis buffer to the sample to lyse any remaining red blood cells and release the RNA.
4. Transfer the lysate to a spin column and centrifuge it to remove any cellular debris.
5. Wash the spin column with a wash buffer to remove any impurities.
6. Elute the RNA from the spin column with an elution buffer.
It is important to follow the instructions carefully to ensure that the RNA isolation is successful and that the resulting RNA is of high quality. The procedure is optimized to achieve reliable results within Approximately **1 hour (24 samples simultaneously).**

Handling Requirements and Safety Information

⚠ All solutions are clear and should not be used when precipitates have formed. Warm the solutions at +15 to +25°C or in a 37°C water bath until the precipitates have dissolved.

⚠ RLT2 Buffer and WR1 Buffer contain guanidinium hydrochloride which is an irritant.

⚠ß-Mercaptoethanol (ß-Me) is hazardous to human health. perform the procedures involving ß-Me in a chemical fume hood.

⚠ Do not allow RLT2 Buffer and WR1 Buffer to touch your skin, eyes, or mucous membranes. If contact does occur, wash the affected area immediately with large amounts of water.

⚠ If you spill the reagent, dilute the spill with water before wiping it up.

⚠ Do not use any modified ethanol.

⚠ Do not pool reagents from different lot numbers.

⚠ Immediately after usage, close all bottles to avoid leakage, varying buffer concentrations, or buffer conditions.

⚠ After first opening store all bottles in an upright position.

⚠ Do not allow the RLT2 Buffer and WR1 Buffer to mix with sodium hypochlorite found in commercial bleach solutions. This mixture can produce highly toxic gas.

⚠ Wear protective disposable gloves, laboratory coats, and eye protection, when handling samples and kit reagents.

⚠ Do not contaminate the reagents with bacteria, viruses, or nucleases. Use disposable pipets and nuclease-free pipet tips to remove aliquots from reagent bottles.

preparation procedure:

Working Solution Preparation

FPKT032.0025-25 prep

⚠ **Add 2.5 ml of absolute ethanol to WR1 Bottle.**

⚠ **Add 10 ml of absolute ethanol to WR2 Bottle.**

FPKT032.0050-50 prep

⚠ **Add 5 ml of absolute ethanol to WR1 Bottle**

⚠ **Add 20 ml of absolute ethanol to WR2 Bottle**

FPKT032.0100-100 prep

⚠ **Add 10 ml of absolute ethanol to WR1 Bottle.**

⚠ **Add 40 ml of absolute ethanol to WR2 Bottle.**

⚠Please note that the Ethanol concentration of a Washing Buffer may decrease during long-term storage resulting in a drop-down of the final RNA yield.

⚠ before starting Incubate the **ER buffer at 55 to 65 ° C** until the end of the protocol to obtain the maximum yields.

Protocol

1. Add 1 ml RLT1 Buffer to the 2 ml microtube and **add 500 µl Blood** sample. Mix gently (don't Vortex) and centrifuge at maximum speed for 1 minute.

2. Discard the supernatant and **add 1ml RLT1 Buffer** again. Mix gently (don't Vortex) and **centrifuge at maximum speed for 1 minute.**

3. Discard the supernatant and **Add 400 µl RLT2 Buffer** and **3.5 µl of ß-Mercaptoethanol** and Vortex vigorously for **1 min** to resuspend the cells completely. Incubate at room temperature for 5 min.

4. Add 600 µl Absolute ethanol to the homogenized lysate, and mix well by pipetting.

⚠If the clump is still visible after the vortex, pipet the sample mixture up and down to break down the clump.

5. To transfer the sample to a High Pure Filter Tube:
-Insert one High Filter Tube in one Collection Tube.
-Pipet the entire sample into the upper reservoir of the Filter Tube **(max. 800 µl).** Insert the entire High Pure Filter Tube assembly into a standard tabletop centrifuge. Centrifuge the tube assembly for **1 min at ≥8000 xg (≥10,000 rpm).**

6. Optional Step: DNase I digestion to eliminate genomic DNA contamination follow the step from 6a. Otherwise, proceed to step 7 directly.
6a. Discard the flow through. **Add 250 µl of WR1** to the Filter Tube, and **centrifuge for 30 sec at ≥8000 xg** (≥10,000 rpm).
6b. Discard the flow-through liquid. For each sample, pipette **5 µl of 10x DNase incubation buffer** into a new sterile microtube and **add 5 µl of DNase I**.
with DEPC-treated water **reach to a volume of 50 µl**, then pipette the solution into the upper reservoir of the filter tube. **Incubate for 15 min at +15 to +25°C.**
6c. Discard the flow through. **Add 250 µl of WR1** to the Filter Tube, and **centrifuge for 30 sec at ≥8000 xg (≥10,000 rpm).**

7. Discard the flow through. **Add 500 µl WR1** to the upper of the Filter Tube and **centrifuge for 30 sec at ≥8000 xg (≥10,000 rpm).**

8. Discard the flow through. **Add 500 µl WR2** to the upper of the Filter Tube. **centrifuge 30 sec at ≥8000 xg (≥10,000 rpm)**. and discard the flow through.

9. Leave the tube assembly in the centrifuge and spin it for **3 min at maximum speed** (approximately 14,000 rpm) to remove any residual Wash Buffer.

10. Discard the Collection Tube and insert the Filter Tube into a clean, sterile RNase-free and DNase-free **1.5 ml microcentrifuge tube.**

11. Add 50 µl ER Buffer to the upper of the Filter Tube. Centrifuge the tube assembly for **1 min** at ≥8000 xg (≥10,000 rpm).
⚠ to obtain the maximum yields you can add the RNA solution to the top of the filter tube and repeat Centrifuge the tube assembly **at ≥8000 xg (≥10,000 rpm).**

The microcentrifuge tube now contains the eluted Cell RNA. Either use the eluted RNA directly in RT.PCR or store the eluted RNA at −20°C for later analysis.