



KIAGENE FANAVAR

KIARNA

High Pure Cell and Tissue RNA extraction kit

Cat. No:

FPKT030.0025

FPKT030.0050

FPKT030.0100

Contents:

Components	25 preps	50 preps	100 preps
LCT1 buffer	5 ml	10 ml	20 ml
LCT2 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
- β-Mercaptoethanol
- Standard tabletop microcentrifuge capable of 13,000 x g centrifugal force

- Microcentrifuge tubes, 1.5 ml, sterile
- liquid nitrogen & mortar
- a rotor-stator homogenizer or a 20-G needle syringe

Application

The High Pure RNA Isolation Kit is designed for the purification of total RNA from Tissue and cultured cell. Due to the integrated DNase digestion step, contamination of the isolated RNA with residual genomic DNA is mostly avoided. In addition, RNA is suited for other techniques like northern blotting, RNase protection and primer extension. 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.

⚠ LCT2 Buffer and WR1 Buffer contain guanidinium hydrochloride which is an irritant.

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

⚠ Do not allow LCT2 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 in order to avoid leakage, varying buffer concentrations or buffer conditions.

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

⚠ Do not allow the LCT2 Buffer and WR1 Buffer to mix with sodium hypochlorite found in commercial bleach solutions. This mixture can produce a 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, virus, or nucleases. Use disposable pipets and nuclease free pipet tips only, to remove aliquots from reagent bottles.

preparation procedure:

Working Solution Preparation

FPKT030.0025-25 prep

⚠ Add 2.5 ml absolute ethanol to WR1 Bottle.

⚠ Add 10 ml absolute ethanol to WR2 Bottle.

FPKT030.0050-50 prep

⚠ Add 5 ml absolute ethanol to WR1 Bottle

⚠ Add 20 ml absolute ethanol to WR2 Bottle

FPKT030.0100-100 prep

⚠ Add 10 ml absolute ethanol to WR1 Bottle.

⚠ Add 40 ml 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.

Contact and Support: If you have questions or experience problems with Kiagene Fanavar products, please contact our Technical Support staff. Our scientists are committed to providing rapid and effective help. Website: www.kiagene.ir Email: Techsupport@kiagene.ir Tel: 02191010809



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Protocols

1. Collect 1 ~ 5 ×10⁶ cells by centrifuge at 300 x g for 5 min at 4 °C. Remove all the supernatant. For Tissue Sample **Weight up to 30 mg of tissue sample**. Grind the sample in liquid nitrogen to a fine powder with a mortar and transfer the powder to a new microcentrifuge tube (not provided).

⚠️ Avoid thawing the sample during weighing and grinding.

2. Resuspend cells in 200 µl LCT1 Buffer. Don't add this Buffer to tissue Samples.

⚠️ Do not overload, too much sample will make cell lysis incompletely and lead to lower RNA yield and purity.

3. Add 400 µl LCT2 Buffer and 3.5 µl of β-Mercaptoethanol and Vortex vigorously for **1 min** to resuspend the cells completely. For Tissue Sample Homogenize the sample by using a rotor-stator homogenizer or by passing the sample lysate through a 20-G needle syringe 10 times. 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 vortex, pipet 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 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 **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 8 directly.

6a. Discard the flow through liquid. **Add 250 µl of WR1** to the Filter Tube, **centrifuge 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 liquid. **Add 250 µl of WR1** to the Filter Tube, **centrifuge 30 sec at ≥8000 xg (≥10,000 rpm)**.

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

8. Discard the flow through liquid. **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.

Contact and Support: If you have questions or experience problems with Kiagene Fanavar products, please contact our Technical Support staff. Our scientists are committed to providing rapid and effective help. Website: www.kiagene.ir Email: Techsupport@kiagene.ir Tel: 02191010809