KIAGENE FANAVAR

MEGARENA
High Pure Total RNA extraction kit

Cat. No:

FPKT029.0025

FPKT029.0050

FPKT029.0100

Contents:

Components	25 preps	50 preps	100 preps
LR 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
Column and collection tubes	25 each	50 each	100 each

Kit storage:

⚠ This kit should be stored at room temperature. 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

For Isolation of Total RNA from Cultured Cells:
- PBS

For the isolation of total RNA from human blood:
- Red Blood Cell Lysis Buffer (RLT)
- PBS

For the isolation of total RNA from Animal Tissue:
- liquid nitrogen & mortar
- a rotor-stator homogenizer or a 20-G needle syringe

For the isolation of total RNA from yeast:
- Lyticase or zymolase
- PBS

For the isolation of total RNA from bacteria:
- Lysozyme (Lysozyme reaction solution: (10mg/ ml lysozyme; 20mM Tris-HCl, pH 8.0; 2mM EDTA; 1.2% Triton))

For the isolation of Total RNA from Paraffin-embedded tissue
- xylene

Application

The High Pure RNA Isolation Kit is designed for the purification of total RNA from cultured cells. Other sample materials like Animal Tissue, blood, yeast, and bacteria require an additional specific pre-lysis treatment, which is described in the **Additional Equipment and Reagent required**. 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.

⚠ LR 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 LR 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 LR 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

FPKT033.0025-25 prep

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

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

FPKT033.0050-50 prep

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

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

FPKT033.0100-100 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.

Protocols

Isolation of Total RNA from Cultured Cells (suited for 1~ 5× 10^6 cells)

1. Collect 1 ~ 5 ×10^6 cells by centrifuge at 300 x g for 5 min at 4 °C. Remove all the supernatant.

2. Resuspend cells in **200 µl PBS**.

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

3. Add 400 µl LR Buffer and **3.5 µl of ß-Mercaptoethanol** and Vortex vigorously for **1 min** to resuspend the cells completely.

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. 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.

Isolation of Total RNA from Human Blood (suited for 200 - 500 µl whole blood)

1. Add 1 ml Red Blood Cell Lysis Buffer (RLT: Not Provided) to a sterile 2 ml reaction tube.

2. Add 500 µl human whole blood and mix by inversion.
⚠Do not vortex.

3. Place the tube on a rocking platform or gyratory shaker for **10 min at +15 to +25° C**. Alternatively, manually invert the sample periodically for **10 min**.

4. Centrifuge for 5 min at 500 × g in a standard tabletop centrifuge. With a pipette, carefully remove and properly dispose of the clear, red supernatant.
⚠Do not vortex.

5. Add **1 ml Red Blood Cell Lysis Buffer (RLT buffer)** to the white pellet and mix by "flicking" the tube until the pellet is resuspended.

6. Centrifuge for 3 min at 500 × g. Carefully remove and properly dispose of the supernatant, particularly the red ring of blood cell debris that forms around the outer surface of the white pellet.

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7. Resuspend the white pellet in **200 µl PBS** and follow the protocol Isolation of Total RNA from Cultured Cells from **step 3.**

Isolation of Total RNA from Animal Tissues.

1. 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. Add 350 µl of LR Buffer and **3.5 µl of ß Mercaptoethanol**. 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**.

3. follow the protocol Isolation of Total RNA from Cultured Cells from Step **4**.

Isolation of Total RNA from Yeast. (Suited for 1 × 10^8 cells)

⚠It is recommended to harvest cells during the mid-log or late-log phase of growth (OD600 ≤ 2.0). The cell number can be counted in a hemocytometer chamber or determined by measuring the optical density at 600 nm in a spectral photometer. Use a dilution that gives an A600 of 0.1 - 0.15/ml (0.1 A600 corresponds to approx. 2 × 106 cells.)

1. Collect the sample by centrifugation at **2,000 × g for 5 min** in a standard tabletop centrifuge.

2. Add 10 µl Lyticase (0.5 mg/ml), incubate for 15 min at 30°C.

3. Follow the protocol Isolation of Total RNA from Cultured Cells from Step **3**.

Isolation of Total RNA from Bacteria (gram-positive and gram-negative) (Suited for 1 x 10^9 cells)

1. Collect the sample by centrifugation **at 2,000 × g for 5 min** in a standard tabletop centrifuge. Resuspend the pellet in **200 µl 10 mM Tris, pH 8.0**.

2. Add 4 µl Lysozyme (50 mg/ml), incubate for 10 min at 37°C.

3. Add 400 µl LR Buffer and mix well

4. Combine the High Pure Filter Tube and the Collection Tube and pipette the sample in the upper reservoir. Centrifuge for **15 s at 8,000 × g** in a standard tabletop centrifuge, discard the flowthrough and again combine the Filter Tube and the used Collection Tube.

5. Pipette **40 µl DNase** Incubation Buffer into a sterile reaction tube, **add 10 µl DNase I**, mix and pipette the solution in the upper reservoir of the Filter Tube. **Incubate for 60 min at +15 to +25°C.**

6. Follow the protocol Isolation of Total RNA from Cultured Cells from Step **7**.

Isolation of Total RNA from Paraffin-embedded tissue

1. Transfer up to **15 mg paraffin-embedded tissue** sample to a microcentrifuge tube (not provided). Remove the extra paraffin to minimize the size of the sample slice.

2. Add 0.5 ml xylene, mix well, and **incubate at room temperature for 10 min**.

3. Centrifuge at full speed for 3 min. Remove the supernatant by pipetting.
4. Add 0.25 ml xylene, mix well, and **incubate at room temperature for 3 min.**

5. Centrifuge at full speed for 3 min. Remove the supernatant by pipetting.

6. Repeat step 4 and step 5

7. Add 0.3 ml Absolute ethanol to the deparaffined tissue, and mix gently by vortexing. **Incubate at room temperature for 3 min.**

8. Centrifuge at full speed for 3 min. Remove the supernatant by pipetting.

9. Repeat step 7 and step 8.

10. Follow the Animal tissue Protocol starting from **step 1** for sample disruption then follow the protocol Isolation of Total RNA from Cultured Cells from **step 4**.

RNA Clean-Up Protocol

1. Transfer 100 µl of RNA sample to a microcentrifuge tube.
⚠If the RNA sample is less than 100 µl, add RNase-free water to make the sample volume to 100 µl.

2. Add 300 µl of LR Buffer and **300 µl Absolute ethanol**. mix well by vortexing. transfer the sample to a High Pure Filter Tube.

3. Centrifuge at full speed for 1 min and discard the flow-through.

4. Follow the protocol Isolation of Total RNA from Cultured Cells from Step **7**.