T-Pro FastEZ Purix RNA Extraction kit (40)

Store at RT

(JZ88-T002) 40test/kit

T-Pro Biotechnology

For laboratory research use only. Not recommended for clinical diagnosis.

Product Overview	The T-Pro FastEZ Purix RNA Extraction Kit uses magnetic bead technology to quickly and reliably extract high-quality genomic RNA from animal tissue samples. This product is intended for professional users trained in molecular biology techniques (e.g., technicians and physicians) and is intended for in vitro diagnostic use.
Features	The T-Pro FastEZ Purix RNA Extraction Kit protocol consists of 4 steps (lysis, binding, washing, elution) and is fully automated on the T-Pro Auto 8t mini-system using pre-filled 8-well extraction strips. The process is designed to eliminate the possibility of cross-contamination between samples and allows the safe handling of potentially infectious samples. The sample input volume is 200-300 µl (already lysed in 200 µl tissue lysis buffer), and the elution volume is 100 µl. The purified total RNA can be used in downstream applications such as real-time RT-PCR and next-generation sequencing.
Storage	The T-Pro FastEZ Purix RNA Extraction Kit can be stored at 4°C to 30°C and used until the expiration date printed on the box label. If the kit is stored in a refrigerator, allow it to return to room temperature before use. After extraction, immediately store the RNA at -80°C or lower. Avoid repeated freeze-thaw cycles. When performing downstream analysis, always keep RNA on ice.

Sample	Description
Whole Blood (200-300 µl)	1. Use fresh whole blood or buffy coat for isolation (within 4 hours, keep on ice). Frozen blood samples that have not been treated with any RNA protection agent (e.g., T-Pro RNA stabilizer) are not recommended. Blood samples should be collected in the presence of an anticoagulant; EDTA is preferred, but other anticoagulants such as citrate, heparin, or ACD can also be used. 2. For best results, process blood samples within a few hours of collection and keep at 4°C.
Cultured Cells (10^5–10^6)	1. Cells or isolated blood cells can be collected as a pellet, then snap-frozen in liquid nitrogen and stored at -70°C, or processed immediately. Add T-Pro RNA stabilizer to resuspend the pellet before extraction. 2. After disruption and homogenization, samples can be stored in T-Pro RNA stabilizer at -80°C. Samples frozen in this manner are stable for several months.
Tissue (10-40 mg)	1. Immediately after removal, wash fresh tissue with ice-cold PBS or saline to remove blood, blot dry with sterile filter paper, and cut into small pieces of approximately 5-10 mg. 2. RNA tissue can be first immersed in RNA preservation solution (e.g., T-Pro RNA stabilizer)

	at 4°C overnight and then transferred to -80°C, or snap-frozen in liquid nitrogen immediately. Homogenization and Disruption 1. Liquid nitrogen grinding (recommended): Place tissue pieces in a pre-chilled mortar, add liquid nitrogen, and grind repeatedly to a fine powder. Use a spatula to transfer the powder into a centrifuge tube already containing lysis buffer, then vortex immediately and vigorously. 2. Mechanical homogenization: Place tissue pieces directly into a homogenization tube containing lysis buffer. Use a handheld electric homogenizer (tissue grinder) on ice for no more than 30 seconds at a time to avoid heat-induced nucleic acid degradation. After RNA homogenization, let stand at room temperature for 5 minutes to ensure nucleoprotein separation.

Kit Components

Item	Qty.	
Extraction Strip	40	8-well extraction strip with magnetic beads and buffers
Magnetic Rod	40	magnetic rods for use with the 8t Extractor
Proteinase K Buffer	1	Vial with 0.8 ml (20 mg/ml), for 40 samples
Package Insert	1	Instructions for use for operator

· Treat all samples as potentially infectious and always follow local safety regulations.
· To prevent degradation by intracellular RNases, it is important to snap-freeze tissues in liquid nitrogen and store at -80°C, or process immediately according to the protocol.
· If samples cannot be frozen immediately, treating tissues with an RNA stabilization reagent (e.g., RNALater, Trizol, or T-Pro RNALater solution) is another option to protect RNA. During processing (e.g., weighing), frozen tissue should not be allowed to thaw; keep samples on ice during homogenization.
· After homogenization, use a filter tube or centrifugation to collect clean tissue lysate.

Additional Equipment and Reagents Required

· Standard laboratory equipment
· Pipettes and nuclease-free, aerosol-resistant filter tips
· RNA stabilization reagent: T-Pro RNA later solution (JT90-RO08S)
· RNaseZap (RNase remover)
· DNase (RNase-free grade)
· Red blood cell lysis buffer (RBC lysis buffer)

Limitations and General Precautions

· This product is intended for use by trained personnel only.
· When handling samples and reagents, wear disposable gloves, a lab coat, and goggles. Wash hands thoroughly afterwards.

· All sample handling should be performed in a clean workbench or biosafety cabinet.
· Wear clean gloves, use RNase-free filter tips, and keep the work area, pipettes, and reagents free from viruses, bacteria, and nuclease contamination.
· Cleaning workbench surfaces, equipment, and pipettes with RNaseZap or another nuclease removal solution is one of the simplest methods to remove RNase contamination in the work area.
· Magnetic beads may occasionally appear in the elution buffer. If this occurs, avoid transferring the beads when transferring the extracted product.
· Before and after the extraction procedure, disinfect the T-Pro Auto8t mini-system with 70% ethanol, followed by UV irradiation.
· Do not use the kit after its expiration date.

The elution buffer is pre-filled in the extraction plate. Due to evaporation during the heated elution step of the extraction process, users should expect an initial volume loss of 10-20%.

Sample preparation

RNA Pretreatment: Use RNase-free consumables and reagents throughout. Treat the workbench and tools with an RNase decontaminant. Operate at low temperature (ice bath) and work quickly. Wear gloves and a mask throughout.

Recommended starting amounts: Tissue: approximately 10-40 mg; Cells: 10^5~10^6; Bacteria: approximately 1-2 mL of overnight culture (OD600 ~1-2); Virus solution: depending on titer, take 100-500 µl (process after concentration).

Sample	Procedure
Whole Blood (200-300 µl)	1. Blood RNA is highly unstable. It is recommended to mix blood immediately after collection with an RNA stabilizer (e.g., T-Pro RNA stabilizer) or directly perform white blood cell isolation. 2. White blood cell isolation method: Take 1-2 mL of fresh anticoagulated whole blood, add 5 volumes of red blood cell lysis buffer (RNase-free), let stand on ice for 10-15 minutes until the liquid becomes translucent red. Centrifuge at 400×g for 10 minutes to collect the white blood cell pellet. Wash twice with ice-cold PBS, centrifuge and discard supernatant. Directly add 200-300 µl of lysis buffer from Reagent Well 2 to the pellet, mix to dissolve, and then add the entire mixture back to Reagent Well 2. - Direct lysis method: If blood volume is small (≤100 µl), it can be added directly to Reagent Well 2, but because hemoglobin may affect downstream applications, this is only suitable for a few applications and is not recommended for NGS. - Plasma or serum: Used for cell-free nucleic acid (cfDNA/ctDNA) or viral nucleic acid extraction. Pretreatment is the same as for virus samples; mix directly with lysis buffer without white blood cell isolation.
Tissue (10-40 mg)	• Immediately after removal, wash fresh tissue with ice-cold PBS or saline to remove blood, blot dry with sterile filter paper, and cut into small pieces of approximately 5-10 mg. • RNA tissue can be first immersed in RNA preservation solution (e.g., T-Pro RNA later)

	at 4°C overnight and then transferred to -80°C, or snap-frozen in liquid nitrogen immediately. 1. Trimming and cutting: - Place fresh or frozen tissue on ice or in a petri dish. - Use sterilized scissors or a scalpel to cut the required weight (typically 10-40 mg). - Remove fat or connective tissue as much as possible. - Cut the tissue into pieces as small as possible (approximately 1-2 mm^3). 2. Grinding/homogenization: - Method A (liquid nitrogen grinding – for tough tissue or RNA extraction): - Place tissue fragments into a pre-chilled mortar. Add a small amount of liquid nitrogen. - Grind quickly and forcefully into a fine powder (complete before liquid nitrogen evaporates). - Use a pre-chilled spatula to scrape the powder into a centrifuge tube. - Method B (mechanical homogenization – for DNA extraction): - Place the minced tissue into a centrifuge tube. Add a small amount of PBS or directly add lysis buffer. - Use an electric homogenizer pestle or a plastic pestle to rotate and press up and down inside the tube until the tissue is completely disrupted (no visible particles). 3. Washing (optional): If lysis buffer was not added during grinding and the sample contains blood or impurities, wash once by centrifugation with PBS (e.g., 8,000 rpm, 5 minutes) and remove the supernatant. 4. Take the lysis buffer (200-300 µL) from Reagent Well 2 to dissolve the product from step 2 or 3, then add the entire mixture back to Reagent Well 2.
Cultured Cells (10^5–10^6)	1. Suspension cells: - Transfer the cell suspension to a centrifuge tube, centrifuge (1,000-3,000 rpm, 5 minutes), discard supernatant. - Add 1 mL of sterile PBS to gently resuspend, count, centrifuge again, discard all supernatant. The resulting cell pellet can be used for lysis. Take the lysis buffer (200-300 µL) from Reagent Well 2 to dissolve the cell pellet, then add the entire mixture back to Reagent Well 2. 2. Adherent cells: - Aspirate the culture medium and wash twice gently with PBS. - Add an appropriate amount of trypsin-EDTA to detach cells, stop with serum-containing medium, transfer to a centrifuge tube (1,000-3,000 rpm, 5 minutes), discard supernatant, wash once with PBS, discard supernatant. Special RNA note: If you do not wish to subject cells to trypsin treatment, you can directly add lysis buffer from Reagent Well 2 (200-300 µL) to the culture plate (pre-chilled on ice), lyse the cells, scrape the cells, and then add the entire mixture back to Reagent Well 2.
Bacteria (10^8–10^9)	• Take 1-2 mL of overnight culture, centrifuge at 4,000×g for 5 minutes, discard supernatant, wash once with PBS, centrifuge again to collect the bacterial pellet.

	• For RNA: If bacterial RNA (e.g., transcriptome) needs to be analyzed, it is recommended to treat with 2 volumes of RNA protection reagent (e.g., RNAprotect Bacteria Reagent) before centrifugation, or immediately after centrifugation add lysis buffer from Reagent Well 2 (200-300 µL) to dissolve, then add the entire mixture back to Reagent Well 2. Cell wall disruption (critical step): Bacterial cell wall structures differ; additional treatment is required to ensure effective lysis and that Proteinase K acts efficiently. - Gram-negative bacteria (e.g., E. coli): Dissolve the bacterial pellet directly in lysis buffer from Reagent Well 2 (200-300 µL) and add back to Reagent Well 2. Most will be completely lysed. Optionally, pre-treat with lysozyme (1 mg/mL in 10 mM Tris-HCl, pH 8.0) at 37°C for 15 minutes, then take lysis buffer from Reagent Well 2 (200-300 µL) to dissolve and add back to Reagent Well 2. - Gram-positive bacteria (e.g., Staphylococcus aureus, Streptococcus): Enzymatic pretreatment is mandatory: Suspend the bacterial pellet in 200 µL of TE buffer containing lysozyme (20 mg/mL) and incubate at 37°C for 30-60 minutes. For Staphylococcus, add lysostaphin (25 µg/mL). After treatment, add lysis buffer from Reagent Well 2 (200-300 µL) to dissolve, then add back to Reagent Well 2. - Hard-to-lyse bacteria (e.g., Mycobacterium): Extend lysozyme treatment time and combine with glass bead agitation.

Nucleic Acid Extraction Procedure

1. Carefully remove the foil from the extraction strip.
2. Transfer the lysed sample (200-300 µL) from the centrifuge tube to Well 2 of the extraction strip.
3. Add 20 µL of Proteinase K to Well 2.
4. Place the magnetic rod into Well 1.
5. Select "Door" on the touch panel to eject the tray and place the extraction strip in the designated area. Ensure the magnetic rod is in place. After placing all strips, select "Door" again to fully retract the tray into the instrument.
6. Select "Start" on the touch panel to begin the extraction.
7. Upon completion, select "Door" on the touch panel to eject the tray. Remove the extraction strip from the instrument.
8. Carefully transfer the extracted product (located in Well 7 of the extraction strip) to a nuclease-free microcentrifuge tube.
9. Immediately store the RNA at -80°C or lower. Avoid more than three freeze-thaw cycles. When performing downstream analysis, always keep RNA on ice.

Important Note: Refer to the T-Pro Auto8t mini-system user manual to set up the program on the instrument.