T-Pro FastEZ Purix RNA Extraction kit (40)

Store at RT

(JZ88-T002) 40test/kit

T-Pro Biotechnology

This product is for laboratory research ONLY and not for diagnostic use.

Product Overview	The T-Pro FastEZ Purix RNA Extraction kit uses magnetic bead technology for rapid and reliable extraction of high-quality genomic RNA from animal tissue samples. The product is intended to be used by professional users, such as technicians and physicians, that are trained in molecular biological techniques, and it is intended for in vitro diagnostic use.
Features	The T-Pro FastEZ Purix RNA Extraction kit procedure comprises 4 steps (lyse, bind, wash, elute), with lysis done off-board and 3 steps (bind, wash, elute) carried out fully automated, using pre-filled 8-well extraction plates on the T-Pro Auto 8t mini-system. The procedure is designed to eliminate the potential for sample- to- sample cross-contamination and allows safe handling of potentially infectious samples. Sample input from 200-300ul, which was lysed in 200 µl Tissue Lysis Buffer, and elution volume is 100 µl. Purified total RNA can be used for downstream applications such as real-time RT-PCR, next generation sequencing etc.
Storage	The T-Pro FastEZ Purix RNA Extraction kit can be stored at 4°C to 30°C, and it can be used until the expiration date printed on the box label. If you store the kit in a refrigerator, let it come to room temperature before use. After extraction, store RNA at -80℃ or lower temperature immediately, repeated freeze–thawing is not allowed. Always handle RNA on ice for downstream analysis

Sample	Description
Whole blood or Buffy coat	1. Using the fresh whole blood sample or buffy coat for isolation. (within 4 hr, on ice) Freezing blood samples without any RNA protection treatments are not allowed. The blood sample should be collected in the presence of an anticoagulant, preferably EDTA, although other anticoagulants such as citrate, heparin, or ACD (acid citrate dextrose) can also be used. 2. For optimal results, blood samples should be processed within a few hours of collection and keep in 4°C.
Culture Cells	1. Cells or isolated blood cells can be collected as pellets and either flash-frozen in liquid nitrogen and stored at –70°C, or processed immediately. Add RNA stabilized reagent to resuspend pellet for extraction 2. Alternatively, samples can be stored at –70°C in RNA stabilized reagent after disruption and homogenization. Samples frozen in this way are stable for months.
Tissue	10-40mg animal tissue, homogenized in Trizol or T-Pro RNA Later solution

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, always follow the local safety regulation.

⚠ To prevent degradation by intracellular RNase, it is important that tissues are either flash-frozen in liquid nitrogen and store at –70°C, or process immediately following steps

⚠ Using RNA stabilized reagent (e.g. RNA later, Trizol or T-Pro RNA Later solution) to treat tissue is another option to protect the RNA if the sample cannot be frozen immediately. Frozen tissue should not be allowed to thaw during handling (e.g., weighing), keep sample on ice during homogenization is recommended.

⚠ After homogenization, using filter column or spin to collect clean tissue lysates.

Additional Equipment and Reagents Required

Standard laboratory equipment

Pipettes and nuclease-free, aerosol-preventive filter tips

RNA stabilized reagent: T-Pro RNA Later solution (JT90-R008S), TRIzol RNA Isolation Reagents, RNAlater

RNaseZap

DNase (RNase-free level)

RBC lysis buffer

Limitations and General Precautions

- This product is intended for use by trained personnel only.
- Wear disposable gloves, laboratory coat and eye protection when handling samples and reagents. Wash hands thoroughly thereafter.
- Process all samples on clean bench or in biosafety cabinet.
- Wear clean glove, use RNase-free filter tip, and keep work area, pipettors and reagents free of virus, bacteria and Nuclease contamination
- Using RNaseZap® or other nuclease remover to clean the surface of bench, equipments and pipettors is one of the most easy way to remove the RNase contaminations of work area
- Magnetic beads may occasionally appear in the elution buffer. If so, please avoid the magnetic beads while transferring the extracted product.
- The T-Pro Auto 8t mini-system should be disinfected using 70% Ethanol, followed by UV light, before and after an extraction procedure.
- Do not use the kit after its expiration date.

Elution buffer has been pre-filled in the extraction plate. Users should expect up to 10-20% loss from the initial volume due to evaporation by elution heating in the extraction process.

Sample preparation	
Sample	Procedure
Whole blood	(Protocol 1) 1. Fresh prepare 1x RBC lysis buffer Add ice-cold two volume RBC lysis buffer to one volume blood sample 2. Inverting 3-5 times, incubate on ice for 10-15 min Centrifuge at 1000 x g, 5min, 4℃ 3. Remove supernatant 4. Resuspend pellet with at least 300ul RNA stabilized reagent. Take 300ul mixture for total RNA extraction (Protocol 2) 1. Resuspend whole blood with RNA stabilized reagent at 1:1 ratio. 2. invert the tube 5-10 times for mixing 3. Take 300ul mixture for extraction
Buffy coat	1. Resuspend Purified buffy coat with RNA stabilized reagent at 1:1 ratio. 2. invert the tube 5-10 times for mixing 3. Take 300ul mixture for extraction
Tissue	1. Add RNA stabilized reagent to tissue; make sure the sample if completely immersed in buffer. 2. Homogenized tissue by homogenizer 3. Spin down the lysate 4. Centrifuge at 1000 x g , for 5min on 4℃ 5. Transfer 300ul clean lysate supernatant for extraction
Cultured cells	(Protocol 1) Suspension culture 1. Harvest cell culture 2. Centrifuge at 1000xg, 5min on 4℃ 3. Remove supernatant completely 4. Resuspend cell pellet with RNA stabilized reagent 5. Vortex mixing for 10 sec 6. Take 300ul for extraction (Protocol 2) Monolayer culture 1. Trypsinize or scrape the cells 2. Centrifuge at 300xg, 5min on 4℃ to remove trypsin or culture medium 3. Resuspend pellet with RNA stabilized reagent 4. Vortex mixing for 10sec 5. Take 300ul for extraction
DNA-free RNA extraction	
Trizol	1. Follow the user guide of Trizol to handle sample 2. Perform lyse sample and separate phases 3. Transfer the clean upper aqueous phase containing the RNA to a new tube with T-Pro RNA protect reagent 4. pipet mix several times and transfer 200-300ul for extraction

Perform extraction

- Carefully remove the aluminum foil from the strip
- Transfer 200-300ul **lysate sample** from the tube **to well 2** of the extraction strip.
- **And 20ul proteinase K to well 2**
- Place magnetic rod to well 1.
- Select ”Door” on the touch panel to push out the plate tray and place the extraction strip(s) in the designated area(s). Make sure the magnetic rod were. After all the strip(s) were placed, select “Door” again to return the tray completely back into the instrument.
- Select “Start” on the touch panel
- Start the extraction run.
- After completion, select ”Door” on the touch panel to push out the plate tray.
- Remove the extraction strip(s) from the instrument.
- Carefully transfer the **extracted product**, located **in well7** of the extraction strip, into nuclease-free microcentrifuge tubes.
- Store RNA at -80℃ or lower temperature immediately, repeated freeze–thawing more than three times is not allowed. Always handle RNA on ice for downstream analysis

Nucleic Acid Extraction Procedure

Important note:

Please refer to the User Manual of the T-Pro Auto 8t mini-system to set up the protocol on the instrument.