T-Pro FastEZ Purix DNA Extraction kit (40)

Store at RT

(JZ88-T001) 40test/kit

T-Pro Biotechnology

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

Introduction	Magnetic bead-based nucleic acid extraction technology is a highly mainstream and efficient nucleic acid isolation and purification technique in molecular biology. It utilizes solid-phase carriers (magnetic beads) to selectively adsorb nucleic acids and, with the aid of magnetic separation technology, can quickly extract high-purity DNA or RNA from complex biological samples. Compared to traditional phenol-chloroform extraction, the magnetic bead method offers advantages such as automation, speed, safety, and high throughput. It eliminates cumbersome centrifugation steps, can be integrated with automated workstations, and is widely used in clinical diagnosis, forensic identification, and food testing.
Principle	The core of the magnetic bead method relies on the reversible binding of nucleic acids to the surface of magnetic beads under specific conditions. Commercially available magnetic beads typically have a core-shell structure: the interior is composed of magnetic material (e.g., Fe_3O_4), and the exterior is coated with a modified layer, such as silica hydroxyl groups or carboxyl groups. The principle is based on the following points: · Hydrophobic interactions and hydrogen bonds: In a high-salt, low-pH environment, water molecules in the solution become highly hydrated, causing the hydrophobic portions of dehydrated nucleic acid molecules to be exposed. These then bind to the silica hydroxyl groups on the magnetic bead surface via hydrogen bonds, forming a stable "DNA-magnetic bead" complex. · Electrostatic interactions: The negatively charged phosphate backbone of nucleic acids can bind via electrostatic adsorption to magnetic beads whose surface is modified with cations (e.g., amino groups). By changing the pH or ionic strength, the nucleic acids can be eluted. · Elution principle: When the environment changes to low salt, high pH, or high temperature, the hydrogen bonds or electrostatic forces between the nucleic acid and the magnetic bead are disrupted. The increase in water molecules allows the nucleic acids to rehydrate, thereby releasing them from the magnetic bead surface and returning them to the aqueous solution.
Storage	The T-Pro FastEZ Purix DNA 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.

Sample	Pretreatment methods vary depending on the sample type, but the core goal is to fully lyse the sample and release the nucleic acids:
Whole blood or Buffy coat	Directly use plasma or serum, or first lyse to remove red blood cells if necessary.
Culture Cells	Usually require the use of lysozyme (for bacteria) or repeated freeze-thaw cycles to break down the cell wall/membrane.
Tissue	Must be cut into small pieces first, and then thoroughly disrupted using a grinder or liquid nitrogen.
Viruses	For enveloped viruses, detergents in the lysis buffer can directly disrupt the envelope and release the nucleic acids.

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

Nucleic Acid Extraction Procedure

1. **Cell Lysis (Sample 200-300µl):**

Add the sample to a lysis buffer containing a chaotropic salt (e.g., guanidine hydrochloride) and a detergent. The chaotropic salt disrupts cell membranes, denatures proteins, and inactivates nucleases, while the detergent helps release the nucleic acids.

2. Nucleic Acid Binding:

Add the magnetic bead suspension. In a high-salt environment, nucleic acids specifically adsorb to the surface of the magnetic beads. After thorough mixing, use a magnetic stand to aggregate the magnetic beads against the tube wall, and aspirate the supernatant.

3. Magnetic Bead Washing:

Typically requires 2-3 washes. Add a washing buffer containing alcohol to remove residual proteins, salts, and impurities. Resuspend the magnetic beads, perform magnetic separation again, and discard the waste liquid. Repeat this step to obtain high-purity nucleic acids.

4. Nucleic Acid Elution (50-100µl):

Allow the magnetic beads to air dry briefly to evaporate residual alcohol (to avoid inhibiting downstream reactions). Then add a low-salt elution buffer (e.g., TE or pure water). Slight heating can improve elution efficiency. Finally, use a magnetic stand to move the magnetic beads to the side, and carefully transfer the supernatant containing the purified nucleic acid to a new centrifuge tube.

Storage of Extracted Nucleic Acids

· **Short-term storage:**

DNA can be dissolved in TE buffer (pH 8.0) and stored at 4°C. TE chelates metal ions, protecting DNA from degradation.

· **Long-term storage:**

Both DNA and RNA should be stored in a -20°C or -80°C freezer. RNA is very unstable; it is recommended to perform reverse transcription immediately or store at -80°C, avoiding repeated freeze-thaw cycles.

· **Aliquoting:**

To prevent damage from repeated freeze-thaw cycles, it is recommended to aliquot the nucleic acid into small portions for storage.

Subsequent Experiments

Nucleic acids purified by the magnetic bead method have high purity and ideal OD260/280 ratios (approximately 1.8-2.0), making them suitable for various downstream applications:

· Polymerase Chain Reaction (PCR) / Quantitative Polymerase Chain Reaction (qPCR)
· Next-Generation Sequencing (NGS) Library Construction
· Southern Blot / Northern Blot
· Reverse Transcription Polymerase Chain Reaction (RT-PCR) / Reverse Transcription Quantitative Polymerase Chain Reaction (RT-qPCR) (for RNA samples)
· Site-directed Mutagenesis / Gene Synthesis

Limitations and General Precautions

· This product is intended for use by trained personnel.
· When handling samples and reagents, please wear disposable gloves, a lab coat, and goggles. Wash hands thoroughly afterwards.
· All sample handling should be performed on a clean workbench or in a 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 a 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 Before Loading	
Sample	Operating Steps
Whole Blood (200-300ul)	1. Collect 200-300µL of whole blood using EDTA or sodium citrate anticoagulant tubes. Avoid using heparin (inhibits downstream enzymatic reactions). 2. Red Blood Cell Lysis (Optional): - Add 3-5 volumes of 1X Red Blood Cell Lysis Buffer. Mix gently by inverting or vertexing. - Incubate at room temperature for 5-10 minutes (solution should become translucent red). 3. Separate White Blood Cells: - Centrifuge at 2,000-3,000 rpm for 5-10 minutes. Carefully remove the supernatant. A white blood cell pellet should be visible at the bottom of the tube. If significant red blood cell contamination remains, repeat step 2. 4. Wash (to remove residual lysis buffer): - Add 1 mL PBS or normal saline. Gently pipette or vortex to resuspend the pellet. - Centrifuge again (same parameters as above). Aspirate and discard the supernatant, leaving the pellet. 5. Take the lysis buffer (200µL-300µL) from Reagent Well 2 to dissolve the pellet from step 4, then add the entire mixture back to Reagent Well 2.
Tissue (10-30mg)	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 10-30mg). Remove fat or connective tissue as much as possible. - Cut the tissue into pieces as small as possible (approx. 1-2mm^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 (200µL-300µL) from Reagent Well 2 to dissolve the product from step 2 or 3, then add the entire mixture back to Reagent Well 2.
Culture Cells (10^5~10^6)	1. Collection: Transfer the cell culture medium to a centrifuge tube. Centrifuge at an appropriate speed (e.g., 1,000-3,000 rpm, 5 minutes) and remove the supernatant. 2. Washing: Add 1 mL PBS, gently pipette or vortex to resuspend the cell pellet. Centrifuge again (same parameters), removing the supernatant as completely as possible. 3. Take the lysis buffer (200µL-300µL) from Reagent Well 2 to dissolve the product from step 2, then add the entire mixture back to Reagent Well 2.

Bacteria (10^8~10^9)	1. Culture and Collection: Transfer an appropriate volume of bacterial culture (depending on OD value, typically 1-5 mL) to a centrifuge tube. Centrifuge at 8,000-12,000 rpm for 1-2 minutes. Remove the supernatant culture medium. 2. Washing: Add 1 mL PBS or TE buffer. Vortex to resuspend the bacterial pellet. Centrifuge again (same parameters) and aspirate the supernatant. 3. Enzymatic Cell Wall Disruption (Critical Step): - Gram-negative bacteria (G-): Have a thinner cell wall. Can usually be lysed directly using lysis buffer (containing SDS and Proteinase K). - Gram-positive bacteria (G+): First, add a buffer containing lysozyme (e.g., 20 mg/mL lysozyme in TE buffer). Resuspend the pellet completely. Incubate in a 37°C water bath or incubator for 30-60 minutes (time varies by strain; some may require longer). The goal is to create pores or disrupt the cell wall, forming protoplasts. Centrifuge again (same parameters) and aspirate the supernatant. 4. Take the lysis buffer (200μL-300μL) from Reagent Well 2 to dissolve the product from step 2 or 3, then add the entire mixture back to Reagent Well 2.

Nucleic Acid Extraction Procedure (using T-Pro Auto8t mini-system)

1. Carefully remove the foil from the extraction strip.
2. Transfer the centrifuge tube containing the lysed sample (200μL-300μL) to Well 2 of the extraction strip.
3. Add 20μL 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.

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