PI-BIOTECH
genética avançada

PI-CLEAR
nucleic acid purification kit

Pi-Clear nucleic acid purification kit - Total RNA

User Guide

Research Use Only (RUO). Not for use in diagnostic procedures.

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1. Types of kits

Pi-Clear Nucleic Acid Purification Kit - Total RNA is available in two sizes:

Product	Quantity
Pi-Clear Nucleic Acid Purification Kit - Total RNA	50 extractions
	250 extractions

2. Shipping and storage

All contents of the **Pi-Clear Nucleic Acid Purification Kit - Total RNA** are shipped at room temperature (15-25 °C). Upon receipt, store all contents at room temperature (15-25 °C).

3. Use

For Research Use Only (RUO). Not for use in diagnostic procedures.

4. Object

The **Pi-Clear Nucleic Acid Purification Kit - Total RNA** was developed to extract and purify high-quality total RNA from a wide variety of sources, including cells and animal tissue. The purified total RNA is suitable for use in a variety of applications, as PCR, RT-PCR, hybridization and sequencing.

5. System overview

The system is based in silica membrane (column). The process is carried out in 4 steps:

1) Cell lysis: cell disruption for RNA release in the presence of guanidine salts and addition of ethanol.

2) Binding: the sample is then processed through a Spin column containing a clear silica-based membrane to which the RNA binds.

3) Washing: Any impurities are effectively removed by subsequent washing.

4) Elution: the purified total RNA is eluted in RNase-free water.

At the end of the process, high-quality total RNA is obtained.

6. Kit contents

The components included in the **Pi-Clear Nucleic Acid Purification Kit - Total RNA** are listed below.

Pi-Clear Nucleic Acid Purification Kit - Total RNA Contents	Quantity
	50 extractions
Spin Column - PB1	50 units (spin column + collection tube)
Lysis Buffer - TL	15 mL
Wash Buffer 1 – TP1	50 mL
Wash Buffer 2 – TP2	**OBS**: 80 mL of Ethanol 96-100 % must be added Final volume: 100 mL
Recovery tube (1,5 mL) - PB2	50 units
RNase-free water – PB3	15 mL

7. Equipment and supplies

In addition to the material described in Section 6 (“Kit Contents”):

- Ethanol 96-100%;
- Micropipettes and sterile filter tips (0,5-10µL, 10-100µL, 100-1000µL);
- DNase/RNase-free microtubes.
- Microcentrifuge;
- Vortex;
- Personal protective equipments (gloves, safety goggles and laboratory coat);
- To tissue animals, maybe mortar, pestle and homogenizer are needed.

8. Biological samples

Total RNA extraction kit from cells and tissue animals. Sample should be collected and storage according recommendation of laboratory to molecular tests.

9. Special care

- Molecular biology procedures, such as RNA extraction, reverse transcription, amplification and detection, require qualified technicians to avoid erroneous results, especially due to degradation of RNA contained in samples or contamination of sample by amplification products.
- Use sterile and disposable plastic material.
- Use sterile, disposable and DNase/RNase - free filter tips and microtubes.
- Use disposable and without powder gloves to manipulate reagents and biological sample, to avoid DNase/RNase contamination from skin surface. Change gloves frequently, specially as protocol changes from raw material to purified material.
- Strictly follow the user guide.
- Applying local, regional and federal environmental regulations to discard reagents and biological material.
- In case of accidents with the kit or for more information about biosafety, consult the SDS (Safety Data Sheet) available on the website www.pi-biotech.com.
- Lysis Buffer (TL) contains guanidine salts, which are harmful in skin contact or when inhaled or ingested.
- Do not add bleach (sodium hypochlorite) or acidic solutions directly to sample preparation waste. Due to the presence of guanidine salts, toxic gases and reactive components may occur.

10. Purified RNA Storage

Store your purified RNA at -20 °C when using the RNA within a few hours of isolation. For long-term storage, store your purified RNA at -70 °C.

11. Observation:

- Buffer Lysis **(TL)** quantity may vary according to the amount of starting material as well the type of sample.
- Elution volume: RNA yield is dependent on sample type, size and quality. Depending on your expected RNA yield, and your sample source and starting amount, use between 30 µL - 100 µL RNase-free water (**PB3**) for each elution.

12. Solution Preparation

Prepare Wash Buffer 2 (**TP2**) according to the instructions described below.

Solution	50 extractions
Wash Buffer 2 (TP2)	Add 80 mL of ethanol 96-100%. Homogenize the solution.

13. Purifying RNA from cells

The protocol was developed to purify RNA from volume of 200 µL of samples. For different volumes, the protocols must be adjusted proportionally. **Before starting the procedure, ensure the preparation of Washing Buffer 2 (TP2), described in Section 12 ("Solution preparation") and 70% alcohol.**

13.1 Lysis

Perform the sample lysis steps, according to the sample type.

Sample type	Procedure
Suspension cells ≤1,0 x 10^6	- Transfer cells to a sterile and DNase/RNase-free microtube. - Centrifuge at 2,000 × g for 5 minutes. - Discard the growth medium. - Resuspend the cells in 200 µL PBS buffer. - Add 200 µL Lysis Buffer (**TL**) and vortex. - Add 400 µL 70% ethanol and vortex. - Proceed to the **Binding** step.
Monolayer cells ≤1,0 x 10^6	- Remove and discard the growth medium. - Add 200 µL Lysis Buffer (**TL**) or enough quantity to cover total cell surface. - Slowly homogenize the bottle or culture plate. - Keep at room temperature for 03 minutes. - Transfer the lysate to a sterile and DNase/RNase-free microtube. - Centrifuge at 2,000 × g for 5 minutes at 4°C. - Collect the supernatant and transfer it to a new sterile and DNase/RNase-free microtube. - Add 400 µL 70% ethanol and vortex. - Proceed to the **Binding** step.
Cell pellet ≤1,0 x 10^6	- Add 200 µL Lysis Buffer (**TL**) to cell pellet. - Homogenize by vortexing until the cell pellet is completely dispersed. - Add 400 µL 70 % ethanol and vortex. - Proceed to the **Binding** step.

13.2 Binding

A - Transfer up to 700 µL of the sample to spin column with the collection tube (**PB1**).

B - Centrifuge at 6.800 x g for 1 minute.

C - Discard the flow-through.

D - Repeat steps **A-C** until the entire sample is processed.

13.3 Washing

E - Add 700 µL Wash Buffer 1 (**TP1**).

F - Centrifuge at 12.000 x g for 15 seconds.

G - Discard the flow-through.

H - Add 500 µL Wash Buffer 2 (**TP2**).

I - Centrifuge at 6.800 x g for 1 minute.

J - Discard the flow-through.

K - Repeat steps **H-J**.

L - Centrifuge at 12.000 x g for 2 minutes, to dry the membrane with bound RNA.

M - Discard the collection tube and insert the spin column into a recovery tube (**PB2**).

13.4 Elution

N - Add 30-100 µL RNase-free Water (**PB3**).

O - Incube at room temperature for 1 minute.

P - Centrifuge at 12.000 x g for 2 minutes and discard the spin column.

Q - Store your purified RNA at -20 °C. For long-term storage, keep the purified RNA at -70 °C.

14. Purifying RNA from animal tissues

The protocol was developed to purify RNA from fresh or frozen in nitrogen animal tissue samples with a maximum of 200 mg each extraction. **Before starting the procedure, ensure the preparation of Washing Buffer 2 (TP2), described in Section 12 (“Solution preparation”) and 70% alcohol.**

14.1 Tissue disruption

Tissue disruption can be use mortars and pestles or electronic grinders/homogenizers.

- To use fresh tissues, Lysis Buffer (**TL**) should be added before disruption.
- To use frozen tissues, the disruption should be made and then add Lysis Buffer (**TL**).
- To use fibrous tissues, freeze the tissue in liquid nitrogen, grind it and after nitrogen evaporation, add the Lysis Buffer (**TL**) to the tissue powder.

Fast and complete disruption of tissue during lysis is important to prevent RNA degradation.

14.2 Lysis

A - Transfer tissue to a sterile and DNase/RNase-free microtube.
B - Add Lysis Buffer (**TL**), according the sample size:

Sample size	Lysis Buffer (TL)
≤5,0 mg	300 µL
5,0 – 50,0 mg	500 µL
50,0 – 200,0 mg	500 µL a cada 50,0 mg

C - Proceed tissue disruption, if not already freaked (see Section 14.1).
D - Centrifuge at 12.000 x g for 2 minutes.
E - Collect the supernatant and transfer it to a sterile and DNase/RNase-free microtube.
F - Add absolute ethanol (1,5 x lysate tissue volume) and vortex.
G - Proceed to the **Binding** step.

14.3 Binding

H - Transfer up to 700 µL of the sample to spin column with the collection tube (PB1).

I - Centrifuge at 6.800 x g for 1 minute.

J - Discard the flow-through.

K - Repeat steps **H-J** until the entire sample is processed.

14.4 Washing

L - Add 700 µL Wash Buffer 1 (**TP1**).

M - Centrifuge at 12.000 x g for 15 seconds.

N - Discard the flow-through.

O - Add 500 µL Wash Buffer 2 (**TP2**).

P - Centrifuge at 6.800 x g for 1 minute.

Q - Discard the flow-through.

R - Repeat steps **O-Q** once.

S - Centrifuge the spin column at 12.000 x g for 2 minutes to dry the membrane with bound RNA.

T - Discard the collection tube and insert the spin column into a recovery tube (**PB2**).

14.5 Elution

U - Add 30-100 µL RNase-free Water (**PB3**) to the center of spin column;

V - Incubate at room temperature for 1 minute;

W - Centrifuge at 12.000 x g for 2 minutes and discard the spin column;

X - Store your purified RNA at -20 °C. For long-term storage, keep the purified RNA at -70 °C.

15. DNase I Treatment

RNA extraction using **Pi-Clear Nucleic Acid Purification Kit – Total RNA** may contain DNA traces. If your downstream application requires DNA-free total RNA, we recommend using DNase I kits, amplification grade, available commercially. However, this may result in reduced RNA yield.