PI-BIOTECH
genética avançada

Pi-Clear Nucleic Acid Purification Kit

DNA/RNA Viral

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 - DNA/RNA Viral is available in one size:

Product	Quantity
Pi-Clear Nucleic Acid Purification Kit DNA/RNA Viral	50 extractions

2. Shipping and storage

All contents of *Pi-Clear Nucleic Acid Purification Kit – DNA/RNA* Viral are shipped at room temperature (15 - 25 °C).

Store protected from light and moisture, at room temperature (15-25 °C).

Carrier **(CR)** must be stored at -20 °C after resuspension in **PB3**. Proteinase K **(PK)** must be stored at -20 °C after first use.

3. Use

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

4. Object

The Pi-Clear Nucleic Acid Purification Kit - DNA/RNA Viral was developed to extract and purify high-quality DNA/RNA viral from sputum, liquor, swab, semen, whole blood, blood plasma and blood serum. The purified DNA/RNA is suitable for use in a variety of applications, such 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 DNA/RNA release in the presence of guanidine salts.

2) Binding: selective binding of nucleic acid to the silica membrane..

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

4) Elution: release of nucleic acid from silica membrane, with nuclease free water.

At the end of the process, purified and high-quality DNA/RNA is obtained.

6. Kit contents

The components included in the **Pi-Clear Nucleic Acid Purification Kit – DNA/RNA Viral** are listed below.

Components	Quantity
Spin column – PB1	50 units (spin column + collection tube)
Proteinase K – PK	300 µL
Carrier - CR	350 µg 350 µg (freeze dried reagent)
Lysis Buffer - TL	15 mL
Wash Buffer 1 – TP1	50 mL
Wash Buffer 2 – TP2	**OBS:** 48 mL of Ethanol 96-100 % must be added Final Volume: 60 mL
Tubo coletor (1,5 mL) – PB2	50 unidades
Nuclease-free Water - PB3	15 mL

7. Equipaments and supplies

Material required In addition tol described in Section 6 “Kit contents”:

A. Ethanol 96-100%;
B. Micropipettes and sterile filter tips (0,5-10µL, 10-100µL, 100-1000µL);
C. Sterile and DNase/RNase-free microtubes;
D. Microcentrifuge;

E. Vortex;

F. Personal protective equipments (gloves, safety goggles and laboratory coat).

8. Biological Sample

DNA/RNA Viral kit from sputum, liquor, swab, semen, whole blood, blood plasma and blood serum. 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) and Wash Buffer 1 (TP1) contain 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 DNA/RNA Storage

Store your purified DNA/RNA at -20 °C when using the material within a few hours of isolation. For long-term storage, store your purified DNA/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: DNA/RNA yield is dependent on sample type, size and quality. Depending on your expected DNA/RNA yield, and your sample source and starting amount, use between 30 µL - 100 µL Nuclease-free water (PB3) for each elution.

12. Solution Preparation

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

Solution	Preparation
Carrier (CR)	Add 350 µL of Nuclease-free Water (PB3). Homogenize the solution
Wash Buffer 2 (TP2)	Add 48 mL of ethanol 96-100%. Homogenize the solution.

13. Protocol

13.1 Sample Lysis

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

Sample Type	Procedure
Total blood, blood plasma and blood	- Add 200 µL of sample at room

serum, liquor and semen	temperature into a clean, sterile and DNase/RNase-free microtube; - Add 5 µL of Proteinase K (**PK**) into the microtube and homogenize the sample;. - Add 200 µL of Lysis Buffer (**TL**) and homogenize vigorously in vortex (10-15 seconds); - Proceed to the **Binding** step.
Swab in transport medium / stabilizer	- Press the swab with the tip in transport medium to extract the biological material (can be centrifuge in a brief spin); - Discard the swab and add 200 µL of transport medium with sample into a clean, sterile and DNase/RNase-free microtube; - Add 5 µL of Proteinase K (**PK**) into the microtube and homogenize the sample; - Add 200 µL of Lysis Buffer (**TL**) and homogenize vigorously in vortex (10-15 seconds); - Proceed to the **Binding** step.

13.2 Binding

A - Add 5.6 µL of Carrier (**CR**) previously prepared and homogenize.

B - Incubate at room temperature (15 - 25 °C) for 3 minutes.

C - Add 200 µL of Ethanol (96-100%) to each microtube with sample and homogenize in vortex (10-15 seconds).

D - Incubate at room temperature (15 - 25 °C) for 5 minutes.

E - Transfer up 700 µL of lysate sample to spin column (**PB1**).

F - Centrifuge at 4,000 x g for 3 minutes.

G - Discard the flow-through.

H - If necessary, repeat steps **E-G** until to process the complete sample..

13.3 Washing

I - Add 700µL of Wash Buffer 1 (**TP1**) to each spin column with sample.

J - Centrifuge at 11,000 x g for 1 minute.

K - Add 500µL of Wash Buffer 2 (**TP2**) previously prepared.

L - Centrifuge at 11,000 x g for 1 minute.

M - Discard the flow-through.

N - Repeat steps **K-M**.

O - Centrifuge at 12,000 x g for 2 minutes, to dry the membrane with bound DNA/RNA.

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

13.4 Elution

Q - Add 30~100 µL of Nuclease-free Water (**PB3**) in the center of the membrane.

R - Incubate at room temperature for 3 minutes.

S - Centrifuge at ≥ 15,000 x g for 3 minutes and discard the spin column.

T - Store the purified DNA/RNA at -20°C, if not used immediately. For long-term storage, keep the purified DNA/RNA at -70ºC.