

Pi-Clear nucleic acid purification kit - Total RNA

ATTENTION: Before starting the assay, read the item "Special Care" in "User Guide".

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

Storage: room temperature (15 - 25 °C)

Required materials: Ethanol 96-100% and extra DNase/RNase-free microtubes.

Validated procedure: suspension cells (PBS), monolayer cells, cell pellets ($\leq 1,0 \times 10^6$) and animal tissues.

Procedure

A) Sample Lysis

- 1 Add sample into a clean, sterile and DNase/RNase-free microtube;
Obs: prepare sample as described in "Instruction for Use";
- 2 Add 200 μ L Lysis Buffer (TL) and vortex;
- 3 Add 400 μ L Ethanol 70% and vortex.



Sample

+ 200 μ L TL
vortex
+ 400 μ L Ethanol
70%
vortex


Nucleic acid Extraction and Purification

B) Binding

- 4 Transfer up to 700 μL of the sample to spin column with the collection tube (PB1);
- 5 Centrifuge at 6.800 x g for 1 minute;
- 6 Discard the flow-through;
- 7 Repeat steps 4-6 until the entire sample is processed.

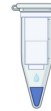


Transfer up
sample to PB1
6.800 x g for 1 min




C) Washing


- 8 Add 700 μL Wash Buffer 1 (TP1);
- 9 Centrifuge at 12.000 x g for 15 seconds;
- 10 Discard the flow-through;
- 11 Add 500 μL Wash Buffer 2 (TP2);
- 12 Centrifuge at 6.800 x g for 1 minute;
- 13 Discard the flow-through;
- 14 Repeat steps 11-13 once;
- 15 Centrifuge the spin column at 12.000 x g for 2 minutes to dry the membrane with bound RNA;
- 16 Discard the collection tube and insert the spin column into a recovery tube (PB2).




+ 700 μL TP1
12.000 x g for 15 sec




+ 500 μL TP2
6.800 x g for 1 min



+ 500 μL TP2
6.800 x g for 1 min



12.000 x g for 2 min




D) Elution


- 17 Add 30-100 μL RNase-free Water (PB3) to the center of spin column;
- 18 Incubate at room temperature for 1 minute;
- 19 Centrifuge at 12.000 x g for 2 minutes and discard the spin column;
- 20 Store your purified RNA at $-20\text{ }^{\circ}\text{C}$.
For long-term storage, keep the purified RNA at $-70\text{ }^{\circ}\text{C}$.



30-100 μL PB3
1 min RT



12.000 x g for 2 min



Access the complete "User Guide"

