



avigene

Molecular Biology Products

AviRex™ Total RNA

for animal tissue, blood and cell culture

Before Starting

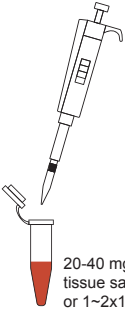
Add 48 ml of absolute ethanol to the PW (only at the first use).

Reagents NOT Provided

1. Chloroform
2. 70% ethanol

Protocol

- 1 Cutting the tissue into the small pieces on a sterile petri dish by a scalpel to increase tissue lysis in the RL solution. Transfer 20-40 mg of tissue (20 mg for liver or spleen) or 150 μ l blood or at least $1\sim 2 \times 10^6$ cells (for cell cultures) into a 1.5 ml tube and add 750 μ l of RL solution.
- 2 Pipetting the tissue into and out of the tip to avoid clumps. You can also homogenize hard tissue by homogenizer on ice. Incubate at room temperature for 5 min.
- 3 Add 200 μ l of chloroform to the mixture. Shake it completely for 15 s and incubate for 3 min at room temperature.
- 4 Spin for 12 min at 13,000 rpm at 4 °C.
- 5 Transfer 400 μ l of the upper phase into a new 1.5 ml tube. Add 400 μ l of 70% (96% ethanol for whole blood samples) ethanol to the mixture and mix them well.
- 6 Transfer mixture to the spin column. Do NOT touch upper rim of column. Spin for 1 min at 13,000 rpm.
- 7 Pour off the flow-through of collection tube.
- 8 Add 700 μ l of PW and spin for 1 min at 13,000 rpm.
- 9 Pour off the flow-through of collection tube. (Optional: repeat step 8 and 9 with 500 μ l of PW to have more pure RNA)
- 10 Spin for 2 min at 13,000 rpm to remove the remaining of the wash buffer. Transfer the spin column to a new 1.5 ml microtube.
- 11 Add 50 μ l of DEPC-treated water, wait 3 min at room temperature or 57 °C (For more yield). If you want more concentration, add less DEPC-treated water (35 μ l).
- 12 Spin for 1 min at 13,000 rpm to elute RNA from the column. Store RNA solution at -70 °C.



20-40 mg of tissue sample or $1\sim 2 \times 10^6$ cells add 750 μ l RL

Pipettage
RT 5 min

200 μ l chloroform
Shake for 15 s
RT 3 min

Spin 12 min, 4 °C



400 μ l 70% ethanol



Spin 1 min



Pour off

700 μ l PW

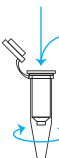
Spin 1 min



Pour off

Spin 2 min

Transfer column



50 μ l DEPC DW

Spin 1 min



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kit content

No.	Description:	Packing	Qty.
1	RL Buffer (RNA Lysis Buffer)	20 ml	2
2	PW Buffer (Wash Buffer)	12 ml	1
3	DEPC-treated Water	3 ml	1
4	Spin Column	50	1
5	Collection Tube	50	1

Research Use Only

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