

Standard Operating Procedure for Total RNA extraction from Kidney Tissue

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Purpose

This protocol describes the required procedure for isolation of total RNA (including micro RNA) from kidney biopsy tissue (needle or wedge) that has been fresh/frozen or stored in RNAlater preservative.

Scope

This procedural format is utilized by the contributing centres to the `SysKID` and `Genome Wide Gene Expression Profiles Associated with Renal Aging` projects. Adherence to the protocol will maximize the quantity and quality of RNA available in human kidney biopsy samples collected for this project.

Materials

QIAGEN miRNeasy Mini Kit (50)
96-100% ethanol
chloroform
Sterile eppendorf tubes
Sterile 20 guage needles
Sterile 1ml syringes
Sterile 10cm petri dishes
Sterile surgical blades

Procedure

The following procedure is a modification of the Qiagen miRNeasy Mini Handbook, October 2007.

Reagent Preparation

If using a new kit a number of reagents need to be supplemented before beginning the extraction.

- Buffers RWT and RPE are supplied as concentrates and require the addition of 96-100% ethanol before first use. The volume of ethanol required is stated on the label of each reagent, ensure the lid of each reagent is marked to indicate that the ethanol has been added.

Disruption of kidney tissue

Place the biopsy tissue onto a sterile petri dish and using a scalpel blade chop the sample into very small fragments, (small enough to pass through a 20 guage needle). If working with a fresh/frozen sample do not allow the sample to thaw. Transfer all of the fragments to a 1.5 ml eppendorf tube containing 700 ul QIAzol lysis reagent (Qiagen).

Homogenization of tissue fragments

Attach a 20 guage needle to a 1 ml syringe and insert through the lid of the tube containing the sample. Draw the sample through the needle to homogenize the sample, repeat a minimum of 10 times or until tissue fragments are no longer visible. Remove the needle, transfer the homogenate to a fresh eppendorf tube and incubate the homogenate at room temperature for 5 minutes. Add 140 ul of chloroform to the homogenate, cap tube securely and shake vigorously for 15 seconds. Incubate

homogenate at room temperature for 3 minute. Centrifuge the samples at 12,000 x g for 15 minutes at 4°C. Retain the upper aqueous phase in a fresh eppendorf tube.

Total RNA purification

- Add 1.5 volumes of 100% ethanol to the sample and mix well by pipetting.
- Transfer 700 ul of the sample to an RNeasy spin column placed in a 2 ml collection tube. Close the lid and centrifuge for 30 seconds at 8000 x g. Discard the liquid that has passed through the filter.
- Load the remaining sample onto the column, centrifuge (30 s at 8000 x g) and discard the flow through liquid.
- Add 700 ul Buffer RWT to the column, close the lid and centrifuge (30s at 8000 x g). Discard the flow through liquid.
- Add 500 ul Buffer RPE, close the lid and centrifuge (30s at 8000 x g). Discard the flow through liquid.
- Add 500 ul Buffer RPE, close the lid and centrifuge (2 min at 8000 x g). Carefully remove the column from the collection tube so that the column does not touch the flow through liquid and place the column into a new 1.5ml collection tube.
- To elute the RNA add 32 ul Rnase-free water directly to the spin column filter and centrifuge (1 min at 8000 x g). Reserve 1 ul of the eluate for quantitation with Nanodrop spectrophotometer and 1 ul for the Bioanalyzer. Freeze the remaining 30 ul of RNA at -80°C until required.