Standard Operating Procedure for RNA and DNA extraction from Kidney Tissue

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Purpose

This protocol describes the required procedure for handling for RNA and DNA isolation from kidney biopsy tissue that has been stored in RNAlater preservative.

Scope

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

Materials

QIAGen AllPrep DNA/RNA Mini Kit (50) Cat.No. 80204
96-100% ethanol
14.3 M β-mercaptoethanol
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 AllPrep DNA/RNA Mini Handbook, November 2005.

Reagent Preparation

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

- Add β-mercaptoethanol (β-ME) to the quantity of RLT buffer that will be used that day at a concentration of 10 ul β-ME per 1 ml of buffer.
- 3 buffers (RPE, AW1 and AW2) 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.
- Prepare a solution of 70% ethanol by diluting 96-100% ethanol with Rnase free water; 600 ul per sample will be required.

Disruption of kidney tissue

Remove the biopsy from the RNAlater solution onto a sterile petri dish. Using a scalpel blade chop the sample into very small fragments, (small enough to pass through a 20 guage needle). Transfer all of the fragments to a 1.5 ml eppendorf tube containing 600 ul RLT buffer (Qiagen) that has been supplemented with β-ME.
NOTE – handle solutions containing β-mercaptoethanol in a fume hood.

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 and centrifuge the samples at top speed for 3 minutes to pellet

out tissue debris. Carefully collect the supernatant, this lysate contains the DNA and RNA. Transfer the lysate to an AllPrep DNA spin column (blue) placed in a 2ml collection tube. Centrifuge at 8000 x g for 30 seconds or until all liquid has passed through the filter. Place the AllPrep DNA spin column into a new 2 ml collection tube and store at room temperature until ready for the DNA purification steps. Use the liquid that has passed through the filter for the RNA purification.

Total RNA purification

- Add an equal volume of 70% ethanol to the liquid that has passed throught the AllPrep DNA spin column (usually around 560 ul) and mix well by pipetting.
- Transfer 700 ul of the sample to an Rneasy spin column (pink) placed in a 2 ml collection tube. Close the lid and centrifuge for 30 seconds at 8000 xg. Discard the liquid that has passed through the filter.
- Load the remaining sample onto the column, centrifuge (30 s at 8000 xg) and discard the flow through liquid.
- Add 700 ul Buffer RW1 to the column, close the lid and centrifuge (30s at 8000 xg). Discard the flow through liquid.
- Add 500 ul Buffer RPE, close the lid and centrifuge (30s at 8000 xg). Discard the flow through liquid.
- Add 500 ul Buffer RPE, close the lid and centrifuge (2 min at 8000xg). 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 xg). Reserve 1 ul of the eluate for quantitation with RiboGreen and 1 ul for the Bioanalyzer. Freeze the remaining 30 ul of RNA at -80^{o}C until required.

Genomic DNA purification

- To the AllPrep DNA spin column add 500 ul Buffer AW1, close the lid and centrifuge (30 s at 8000 xg). Discard the flow through liquid.
- Add 500 ul Buffer AW2 to the column, close the lid and centrifuge (2 min at 16000 xg). 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 DNA place the AllPrep DNA spin column in a new 1.5 ml collection tube. Add 73 ul Buffer EB directly to the spin column filter and close the lid. Incubate at room temperature for 1 minute, then centrifuge for 1 minute at 8000 x g to elute the DNA. Reserve 3 ul of the eluate for quantitation with PicoGreen. Freeze the remaining 70 ul of DNA at -80^{o}C until required.