June 22, 04

[Qiagen] RNeasy (Micro) Kit

- Add 10µl Mercaptoethanol/1ml RLT (RLT is stable for a moth at RT after addition of mercaptoethanol).
- Add 4Vol of absolute ethanol to RPE buffer.
- Dissolve DNase in 550 µl of RNase free water. Do not vortex (as Dnase is physically sensitive). Aliquote and store at -20C.
- Use carrier RNA when the starting material is less than 10 µg. Use carrier DNA and the sample +RLT before homogenization.
- Dissolve carrier RNA (310 µg) in 1ml RNase free water (store at -20C).

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(1) Add **350 µl** of **RLT** (mercaptoethanol added) to your sample prior to homogenization. If the sample is <10 µg tissue, add 20ng (i.e. 5 µl of 4g/ µl) of carrier RNA
(2) Spin at >10,000 rpm for 3min. Transfer the supernatant to a new tube (i.e. the lysate).
(3) Add **350 µl** (i.e. 1 vol) of 70% **ethanol**. Pipet up and down but **do not centrifuge**. If ppt appears after ethanol adding, worry free about it.
(4) Apply the sample (with any ppt) to **RNeasy spin column** in 2ml collection tube. Spin for 15second at >8000 xg (i.e. >10,000 rpm) and discard the flow-through.
(5) Add 1 vol **RW1** buffer, spin (15sec at >8000g).
(6) Prepare **DNase** solution (as **10 µl DNase in 70 µl RDD buffer**). **Do not vortex** but gently pipette up and down. **DNase is physical sensitive**. Take 80 µlto RNase column and incubate for 15min at RT. Mix well both DNase/RDD + silica gel membrane so the rxn takes place.
(7) Add **350 µl RW1** (after genomic DNA digestion). Centrifuge for 15s/8000 xg
(8) Transfer the silica column in a new 2ml collection tube. Pipet **500 µl**of **RPE buffer** (ethanol added) and spin 15s/8000 xg.
(9) Add **500 µl** 80% ethanol and spin for 2min (2min/ > 8000 xg). Remove RNeasy column carefully from the collection tube, so it does not touch the ethanol.
(10) Transfer the column to a new 2ml collection tube. Open the cap and spin at the highest speed for 5min. Discard the flow-through (to insure that all ethanol is gone).
(11) **Elution step:** Transfer the silica column to a new 1.5ml tube and add **14 µl of RNase free water**. Close the tube and spin for 1min at maximum speed (you can use less vol to have more conc RNA).

This protocol will extract the total RNA but enrich the mRNA, since any RNA < 200 base (i.e. rRNA and tRNA) will be in the flow-through. Any RNA >200 (i.e. mainly mRNA) will be trapped in the silica gel.

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