RNAclean： RNA Purification Kit

I. Kit Content, Storage, and Stability

Content	Storage	50preps (RP1802)
Buffer RC	RT	20 ml
Buffer RE	RT	30 ml
Buffer RW	RT	15 ml
		Add 60 ml ethanol before use
RNase-free H_2O	RT	10 ml
RNase-free Spin-column AC	RT	50
Collection Tube (2ml)	RT	50

All reagents will be stable for 12 months if stored properly.

Reminder:

1． ***Add ethanol to Buffer RW before use, mix adequately, and then check the box on the label showing ethanol was added!***

2． Buffer P2 may precipitate under low temperature. Incubate at 37°C for a few minutes **until buffer is clear**, and then let the buffer cool to RT before use.

3． Keep the reagent lids tightly caped when not in use to prevent evaporation, oxidation, and changes in pH.

II. Principle

This kit is designed for purifying RNA from all kinds of reaction systems (such as DNase digestion, proteinase digestion, and RNA marker). RNA selectively binds to silica membrane in high concentrated salt solution. A serial of elution-centrifugation steps will remove cellular metabolite and proteins etc. Finally, total RNA is eluted from silica membrane in low salt RNase-free water. The purified RNA without any protein contamination can be applied for Northern Blot, Dot Blot, mRNA Isolation, cDNA Synthesis, Primer Extension, mRNA Differential Display etc. The yield of RNA is up to 50μg.

III. Procedure

- ***Add absolute ethanol to Buffer RW before use and check the box on the bottle to indicate the ethanol has been added!***

1. Add RNase-free water to make the total volume of RNA sample up to 100 μl, then add 350 μl Buffer RC and mix gently.
2. Add 50 μl ethanol and mix gently. A flocculated precipitate may occur.
3. Transfer solution and the flocculated precipitate from last step into a Spin-column AC (place the spin-column to collection tube), then centrifuge at 10,000 rpm for 45 seconds. Discard the flow-through**.**
4. Add 500 μl buffer RE and centrifuge at 12,000 rpm for 45 seconds. Discard the flow-through.
5. Add 700 μl buffer RW and centrifuge at 12,000 rpm for 45 seconds. Discard the flow-through.
6. Add 500 μl buffer RW and centrifuge at 12,000 rpm for 45 seconds. Discard the flow-through.
7. Place Spin-column AC back to collection tube，centrifuge at 12,000 rpm for 2 minutes to remove the residual fluid (that may inhibit the downstream reactions).

8．Transfer the Spin-column AC to a new RNase-free tube. Add 50-80 μl RNase-free water (preheated to 65-70℃), and then cool down to room temperature for 2 minute and centrifuge at 12,000 rpm for 1 minute.

9． (Optional) add additional 30 μl RNase-free water in the spin-column, and cool down to room temperature for 2 minutes. Centrifuge at 12,000 rpm for 1 minute.

The volume of elution buffer could be adjusted according to needs. Appropriately reduce elution volume can increase concentration. The minimum volume is 30μl, and less than 30 μl will decrease the elution efficiency and the RNA yield

10. Store RNA at -20℃ or apply to down-stream reactions.