User Manual:

Read all the procedures before using Transcript Purification Kit!!

1. Quick spin transcription reaction tube at 10,000rpm for 30 sec.
2. Add 3 volumes of **Buffer I** (RNA binding buffer) and mix well.
3. Load all the solution into column and wait for the first solution drop at the tip of the column.
4. Spin the tube at 2,000rpm for 1 min (for maximal binding) and then at 10,000rpm for 1min.
5. Add 750μl of **Buffer II** into the column and wait for the first solution drop at the tip of the column.
6. Centrifuge at 10,000rpm for 1 min and discard the solution.
7. Centrifuge at 12,500rpm (or maximal speed) for additional 1min to remove remaining Buffer II.
8. Remove the column cartridge from tube and transfer into a new 1.5ml collecting tube.
9. Air-dry for 5min to completely remove remaining ethanol.
10. Add 40-50μl of DEPC water onto the membrane.
11. Centrifuge at >10,000rpm for 1 min to elute RNA transcripts.
12. Check the quality of purified RNAs with agarose gel electrophoresis and spectrophotometer.

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Notes:

1. **Do not discard** purification columns!!! They can be re-used after treated with Column Activator.
2. Add 400μl of **Column Activator** into the column and incubate for 10-15 min.
3. Add 1min of di-water to the column and let solution drop from the tip naturally.
4. After 10-15 washes, spin the column at >10,000rpm for 2 min to completely dry membrane.
5. The column can be used again without any contaminations (exactly like a new column).
6. See **Column Activator** in this website for more important.

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Troubleshooting

1. Degraded transcripts
   - Transcription reaction failed
     - Column purification itself will not cause RNA degradation. Take 2μl of transcription solution and run in a 1.0-1.5% denatured agarose gel to see if RNAs are successfully synthesized.

   - Contaminated buffer
     - Make sure that Buffer I and Buffer II are not contaminated with RNAses or any salts such as Na^{+} and Mg^{++}. Use new tips for taking Buffer I and Buffer II.

2. Toxic effect of purified RNA transcripts in microinjection study

   Cell death after after microjection of purified RNA transcripts
   - The RNA transcripts may contain remaining ethanol from Buffer II. Use ethanol precipitation to clean RNA transcripts again to remove remaining ethanol from RNA transcripts.
   - The RNA transcripts may be contaminated with proteins and other high concentration salts. The salts and proteins are not removed from transcripts during purification process.
   - The concentration of microjected RNA transcripts is too high. Check the concentration of RNA transcripts before microinjection.