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# RNAclean RNA Purification Kit

**Product Number: RNK1402**

## Shipping and Storage

1. All solutions should be clear. If the ambient temperature is low, the solution may form precipitates and should not be used directly. It can be heated in a 37℃ water bath for a few minutes to restore clarity.
2. To avoid volatilization, oxidation, and pH changes caused by prolonged exposure of reagents to the air, each solution should be covered tightly in a timely manner after use.

## Component

| Component | Storage | RNK1402 50preps |
|---|---|---|
| Buffer RC | RT | 20 ml |
| Buffer RW | RT | 10 ml |
| RNase-free H2O | RT | 10 ml |
| RNase free adsorption column RA | RT | 50 个 |
| Collection tube (2ml) | RT | 50 个 |

## Description

This reagent kit uses centrifugal adsorption columns and silicon matrix membranes, all of which are specially imported adsorption membranes. The difference in adsorption capacity between columns is minimal, and the repeatability is good. Under high salt conditions, RNA binds efficiently and specifically to the silica gel adsorption membrane, while maximizing the removal of proteins, inorganic salt ions, and many organic impurities. Under low salt conditions, RNA is eluted. The amount of RNA samples that can be processed can reach up to 50μg. This kit is used for purifying and recovering RNA from enzyme reaction solutions (such as DNase treatment, protease treatment, RNA labeling, etc.), and can also be used for purifying RNA extracted from other methods. The purified total RNA is free of protein contamination, and the obtained RNA can be used for Northern blot, Dot blot, mRNA extraction, cDNA synthesis, primer extension, differential display, etc.

## Protocol

Tip: 1) Before the first use, please add the specified amount of ethanol to the Buffer RW bottle. After adding, please mark the added ethanol with a check mark in a timely manner to avoid adding it multiple times!

2) All the following steps can be performed at room temperature, but they should be performed quickly to reduce the chance of RNA degradation.

1. Add RNase free water to supplement the RNA sample on ice to 100μl, add 350μl Buffer RC, and mix well.
2. Add 250μl of anhydrous ethanol, mix well without centrifugation.
3. The solution obtained in the previous step, along with any possible precipitates, is transferred to the adsorption column RA (the adsorption column is placed inside the collection tube), centrifuged at 4℃ and 12000 rpm for 45 seconds, and the waste liquid in the collection tube is discarded. The adsorption column is then re placed in the collection tube.

   **If trace DNA residues need to be removed, they can be directly digested on a DNA enzyme column after this step, as detailed in the appendix.**
4. Add 0.5ml of Buffer RW (please check if ethanol has been added first), centrifuge at 4℃ and 12000 rpm for 45 seconds, and discard the waste liquid.
5. Add 0.5ml of Buffer RW, centrifuge at 4℃ and 12000 rpm for 45 seconds, and discard the waste liquid.
6. Centrifuge at 4℃ and 13000 rpm for 2 minutes to remove Buffer RW as much as possible to prevent residual ethanol from inhibiting downstream reactions.

7. Take out the adsorption column RA and place it in an RNase free centrifuge tube. Add 50-80μl of RNase free water to the middle of the adsorption membrane according to the expected RNA production (heating in a 65-70℃ water bath beforehand is more effective). Leave at room temperature for 2 minutes and centrifuge at 12000 rpm for 1 minute. If more RNA is needed, the obtained solution can be re added to the centrifuge adsorption column and centrifuged for 1 minute, or an additional 30μl of RNase free water can be added and centrifuged for 1 minute, and two elutions can be combined.

**The larger the elution volume, the higher the elution efficiency. If a higher RNA concentration is required, the elution volume can be appropriately reduced, but the minimum volume should not be less than 30μl. If the volume is too small, the RNA elution efficiency will be reduced and RNA production will be reduced.**

## Appendix: DNase I column digestion

This kit can also perform DNA enzyme digestion on a centrifuge column to remove trace amounts of DNA contamination from RNA samples. If strict mRNA expression analysis, such as fluorescence quantitative PCR, is required, various commercial RNase free DNases can be purchased to directly digest DNA on a centrifuge adsorption column RA, and then pure RNA can be washed off and used directly. Customers can purchase Buffer RW1 from our company as needed.

Taking RNK3401-DNase Digestion Kit as an example

1. Preparation of DNase I working solution:
   1.1. Gently blow and mix 45μl DNase I buffer and 5μl RNase free DNase I centrifuge tubes to form a working solution (prepare the working solution by scaling up multiple centrifuge columns in proportion).

   **Note: If excessive residual DNA leads to incomplete digestion, the use of enzymes can be increased proportionally to improve digestion efficiency (such as 90μl DNase I buffer and 10μl RNase free DNase I).**
2. Operation steps:
   2.1. Follow the normal steps before, and after completing step 3, follow the following steps.
   2.2. Add 350μl Buffer RW1 to the adsorption column RA, centrifuge at 12000 rpm for 30 seconds, discard the waste liquid, and place the adsorption column in the recovery header.
   2.3. Add 50μl of DNase I working solution to the center of the adsorption column RA and let it stand at room temperature (20-30℃) for 15 minutes.

   **Pay attention to directly drop the working liquid onto the center of the membrane, and do not let the working liquid drop onto the O-ring or centrifuge column wall.**
   2.4. Add 350μl of deproteinized solution RW1 to the adsorption column RA, centrifuge at 12000rpm for 30-60 seconds, discard the waste liquid, and place the adsorption column in the recovery manifold.
   2.5. Follow up on buffer RW steps and other related steps. If it is a reagent kit from another company, proceed with the final rinsing solution rinsing and other subsequent steps.