TIANGEN®

RNAclean Kit

For RNA cleanup and concentration with small elution volumes

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RNAclean Kit

(Spin Column)

Cat. no. 4992728

Kit Contents

Contents	4992728 20 preps
Buffer RK	10 ml
Buffer RW	12 ml
RNase-Free ddH_2O	15 ml
RNase-Free Columns CR2 Set	20
RNase-Free Centrifuge Tubes 1.5 ml	20
Handbook	1

Storage

RNAclean Kit should be stored dry at room temperature (15-25°C) and are stable for 12 months under these conditions.

Introduction

The kit combines the selective binding properties of a silica-based membrane with the speed of microspin technology. RNA could be adsorbed to silica-based membrane efficiently and exclusively in the solution under high salt concentration. At the same time, protein, saline ions, organic impurities and other contaminants could be removed to a large extend. RNA will be eluted in the solution under low salt concentration. Up to 20 μg RNA sample could be processed each.

The kit is used for RNA purification from enzymatic reaction (such as DNase digestion, Protease treatment, RNA labeling and so on). The purified RNA is free of protein contamination, and can be used in many downstream experiments such as Northern blot, Dot blot, mRNA extraction, synthesis of cDNA, primer extension, differential display, etc.

Notes for preventing RNA contamination

1. Wear gloves when handling RNA and all reagents, as skin is a common source of RNase. Change gloves frequently.
2. Use sterilized plastic ware and tips to avoid cross-contamination.
3. Plastic or glass ware should be RNase-free. To wipe off RNase, the glassware could be dried at 150°C for 4 hours, while plastics could be dipped in 0.5 M NaOH for 10 min, and washed by RNA-Free ddH_2O thoroughly and sterilized.
4. Prepare the reagents with 0.01-0.1% DEPC Treated RNase-Free ddH_2O. Treat microcentrifuge tubes and tips with DEPC and autoclave to remove any trace of DEPC before use.

Protocol

β-mercaptoethanol (β-ME) should be added to Buffer RK for the first use. Add 10 µl β-ME per 1 ml Buffer RK. Please prepare the solution fresh right before use. Buffer RK containing β-ME can be stored at 4°C for up to 1 month. Redissolve it before use when precipitate is formed.

Ensure that 96-100% ethanol is added to Buffer RW as indicated on the tag of bottle.

All the steps as below should be performed in a ice bath.

1. Adjust the sample to a volume of 100 µl with RNase-free water. Add 350 µl Buffer RK **(ensure β-ME is added before use)**, and mix well.
2. Add 250 µl 96-100% ethanol to the diluted RNA, and mix well by pipetting. Do not centrifuge. Proceed immediately to step 3.
3. Transfer the sample and precipitate to a Spin Column CR2 placed in a 2 ml collection tube . Close the lid gently, and centrifuge for 30 sec at 12,000 rpm. Discard the flow-through.
4. Add 500 µl Buffer RW **(ensure that ethanol is added into Buffer RW)** to the spin column. Close the lid gently, and centrifuge for 30 sec at 12,000 rpm to wash the spin column membrane. Discard the flow-through.
5. Repeat step 4.
6. Centrifuge for 5 min at 12,000 rpm and discard the flow-through.
7. Place the Spin Column CR2 in a new 1.5 ml collection tube. Add 14-20 µl RNase-Free ddH_2O directly to the center of the spin column membrane. Incubate for 2 min at room temperature. Close the lid gently, and centrifuge for 2 min at 12,000 rpm to elute the RNA.

   Note: Elution buffer volume should be over 14 µl, otherwise RNA yield and quality will be reduced.

   The pH of the elution buffer has great effect on elution efficiency. The pH should be at the range of 7.0-8.5 if water is used as elution buffer, otherwise, efficiency of elution will be reduced. RNA product should be stored at -20°C.
8. It is recommended to repeat step 7 for higher yield , then combine the eluate .