DANAGENE DNA REMOVAL KIT

Ref. 0807 50 preps

1. INTRODUCTION

The DANAGENE DNA Removal Kit provides a method for removal of genomic DNA contamination in RNA preparations using an approach consisting of two sequential filtrations with different MiroSpin columns

DNA contaminating in RNA preparations can serve as a template in PCR to produce a false positive signal from RT-PCR. Although false positives are easily identified by looking at the outcome of a "minus-RT" control.

Features:

- **Efficient removal genomic DNA from RNA preparations using a 2 column process.**
- **Fast protocol: 10 minutes.**
- **RNA is isolated without the use of harmful chemicals as phenol or chloroform.**
- **Efficient isolation of small RNA species using 2 columns process, resulting in minimal contamination of larger RNA and gDNA.**
- **Purified RNA can be used in a number of downstream applications including: qPCR, reverse transcription PCR, Northern blotting, RNase protection and expression array assays.**

2. KIT COMPONENTS

	50 preps	Stockage
Binding Buffer	**25 ml**	Room temperature
Wash Buffer *	**10 ml**	Room temperature
Nuclease-free water	**8 ml**	Room temperature
gDNA removal column	**50 units**	Room temperature
RNA column	**50 units**	Room temperature
Collection tubes	**100 units**	Room temperature

(*) These solutions must be prepared as indicated in the Preliminary Preparations section of the protocol.

The RNA Binding Buffer contains guanidinium thiocyanate which is a potent irritant, wear protective gloves and goggles. Both buffers can form dangerous reactive components when combined with bleach.

3. PROTOCOL

3.1 Preliminary Preparations

- **Add 40 ml of Ethanol 100 % to the Wash Buffer 2**. Keep the container closed to avoid the ethanol evaporation.

3.2 Protocol for cleaning genomic DNA in RNA samples

NOTE: This system does not completely eliminate genomic DNA in those samples whose concentration is very high. In this case dilute the initial sample to minimize the amount to be eliminated. This system also removes a small part of the RNA.

1. **Add 400 µl of Binding Buffer to 100 µl** of the solution containing the RNA **+ 50 µl 100% ethanol.** Mix well by miropipette. If the solution is less than 100 µl, add nuclease-free water. It can also be processed by scaling proportionally the reagents.

2. **Transfer the sample to a gDNA removal column**. Place the spin column in a collection tube.

3. **Centrifuge for 1 minute at 8,000 r.p.m.**

4. **Add 400 µl of 100% ethanol to the supernatant collected in point 3.**Mix well.

5. Take an **RNA column** with its collection tube and add the mixture from point 4. **Centrifuge at 8,000 -10,000 rpm for 60 seconds**. Pass the sample twice as the volume exceeds the capacity of the column.

6. Add **700 µl Wash Buffer**. Centrifuge at maximum speed for 1 minute.

7. **Centrifuge 3 minutes at maximum speed** to remove all the ethanol.

8. Place the RNA column in a new 1.5 ml microtube (not supplied with the kit) to elute the RNA.

9. Elute the RNA in **30-50 µl of Nuclease-free Water.** Incubate 2 minutes and centrifuge at maximum speed for 1 minute. RNA

4. PROBLEM GUIDE AND POSSIBLE ANSWER

For any doubts or additional questions about the protocol, please contact the technical service of DANAGEN-BIOTED S.L info@danagen.es