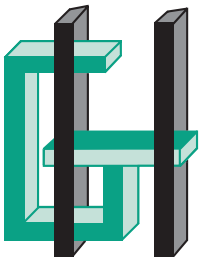
RNApure® Reagent

For convenient extraction of total RNA from tissues or cultured cells

Cat. No. P501, P502, or P503

(for research use only)



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Introduction

The extraction of total RNA from tissues, cultured cells, or other sources is one of the most crucial steps in the differential display process, as it is for other applications such as northern blot analysis, RT-PCR, and DNA microarrays. GenHunter has developed a simple mono-phasic solution for rapid isolation of intact total RNA that is similar to other phenol/guanidine thiocyanate-based RNA isolation products, but **RNApure® has several major advantages**. These include special cell lysis chemicals giving better RNA yield, a yellow color allowing easier visualization during phase separation, and better stability. The high quality total RNA isolated with this product can be used for most any RNA application including differential display, RT-PCR, microarray, northern and reverse northern blot analysis.

Total RNA has several advantages over poly-A RNA for differential display. First, it is much easier to obtain high quality total RNA than poly-A RNA. Second, poly-A RNA is often contaminated with oligo-dT (because of the column extraction method) and therefore often leads to high background on differential display gels. Third, it is much easier to verify the integrity of total RNA than for poly-A RNA.

Please read the entire protocol carefully before using the product!

Precautions

This is a **TOXIC** and **CORROSIVE** reagent (see MSDS form for specifics).

- Avoid exposure to eyes, skin, and mucous membranes.
- Please wear safety goggles and protective gloves at all times during RNA extraction.
- Wash hands thoroughly after handling.
- In case of direct contact, rinse with large amounts of water and seek medical attention immediately.

Liability Waiver: GenHunter shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or in the inability to use this product.

Note: The mRNA Differential Display process is covered by U.S. Patents 5,262,311; 5,599,672; 5,665,547; 5,965,409; Japanese Patent 2843675 and other issued or pending foreign patents licensed to GenHunter Corporation. Purchase of the RNAimage® or RNAspectra® Kits from GenHunter Corporation comes with a sub-license to practice the differential display process.

Instructions

Part 1: Extraction

A) For Cells from Tissue Culture

1) a) If using regular "attached" cells from tissue culture, pour off medium. Set the plate on ice. Move on to step 2.

   b) If using cells growing in suspension, spin down cells and remove the medium. Move on to step 4.

2) Rinse cells with 10 - 20 mL cold Phosphate-buffered saline (PBS).

3) Pour off rinsed PBS. Let plate sit on angle for 1 minute and remove the residual PBS with a 1000 µL Pipette. It is very important to get rid of residual liquid. Otherwise, the ratio of RNApure to cells will be altered, potentially causing a viscous interphase.

4) Add 2 mL of RNApure® per 100 - 150 mm plate to lyse the cells (spread the solution by shaking the plate). Generally, 1-2 mLs is sufficient for 1-10 million cells, but it is better to err on the side of additional RNApure.

5) Let sit on ice for 10 minutes.

6) Pipette the lysate into two 1.5 mL microfuge tubes (label the tubes).

7) Add 150 µL of Chloroform per mL of lysate. Vortex for 10 sec. Freeze the tubes at -80˚C or proceed to the next step.

8) Spin the tubes in Eppendorf centrifuge at 4˚C for 10 minutes. Note: After spinning, no more than 1/2 of the total volume should be in the upper phase. Otherwise, there was too much liquid or not enough RNApure® was added.

9) Carefully remove the upper phase into a clean tube.

10) Move on to Part 2.

B) For Tissues

IMPORTANT NOTE: Tissue samples often contain large amounts of endogenous RNase activity, which can significantly compromise the downstream data generated by any method. Therefore, we highly recommend following the procedure below that includes a second extraction step to completely remove these RNases.

1) Add at least 2-5 mLs of RNApure® to the tissue in a 50 mL conical tube on ice. Ideally, the volume ratio of RNApure® to tissue should be at least 10:1. As a guide, 100 mg ≅ 100 µL. Therefore, 150-200 mg of tissue can be used with 2 mLs of RNApure®.

2) Homogenize the tissue on ice with Polytron™ Homogenizer until the tissue is dispersed.

3) Let sit on ice for 10 minutes.

4) Transfer 1 mL aliquots of the lysate into 1.5 mL microfuge tubes.

5) Spin the tubes in Eppendorf centrifuge at 4˚C for 5 minutes to tissue debris.

6) Remove the cleaned lysate (debris-free) into new 1.5 mL microfuge tubes.

7) Add 150 µL of Chloroform per mL of cleaned lysate. Vortex for 10 sec. Freeze the tubes at -80˚C or proceed to the next step.

8) Spin the tubes in Eppendorf centrifuge at 4˚C for 10 minutes. Note: After spinning, no more than 1/2 of the total volume should be in the upper phase. Otherwise, there was too much liquid or not enough RNApure® was added.

9) Carefully remove the upper phase into a clean tube.

8) Add 1 mL of RNApure® to each upper phase. Vortex for 20 sec.

9) Add 150 µL of Chloroform per mL of above mixture. Vortex for 10 sec. Freeze the tubes at -80˚C or proceed to the next step.

10) Spin the tubes in Eppendorf centrifuge at 4˚C for 10 minutes.

11) Carefully remove the upper phase into a clean tube.

12) Move on to Part 2.

C) For Blood

1) Spin down the blood and remove the plasma.

2) Follow the instructions "For Tissues"above.

Part 2: Purification

1) Add equal volume of Isopropanol and mix vigorously with vortex for 30 seconds. Let sit on ice for 10 minutes.

2) Spin for 10 minutes at 4˚C.

3) Carefully take off the liquid, trying not to disturb the pellet.

4) Rinse the RNA pellet with 1 mL of cold 70% Ethanol. Let set on ice for 5 minutes

5) Spin 2 minutes at 4˚C.

6) Remove the ethanol. Spin briefly and remove the residual liquid with a 200 µL pipette.

7) Resuspend the RNA in 20-50 µL of DEPC-H_2O by gently pipetting up and down until the "jelly-like" RNA dissolves. The volume used can be adjusted based on expected recovery, but RNA should generally be at a concentration of at least 1 µg/µL. Concentrations of 0.5 µg/µL or lower may not store well. Do not use SDS in resuspension if using RNA for differential display or any other PCR application.

8) Measure the concentration by taking 1 µL of the RNA (using P10 pipette) and diluting to 1 mL of H_2O. Read at 260 nm. **1 OD_{260} = 40 µg**

9) Store RNA in aliquots at -80˚C until use.

IMPORTANT NOTE: This procedure produces RNA suitable for northerns and RPA (RNase protections assay). However, since no RNA isolation system can yield RNA free of trace amounts of DNA contamination, if you plan to use your RNA sample for differential display, RT-PCR, or microarrays it is still essential to remove these trace amounts of DNA contamination by DNase I treatment with the MessageClean® Kit (GenHunter Cat # M601).

Appendix I: Expected yield* of total RNA with the RNApure® Reagent

25 mg of tissue or 2.5 x 10^6 cells

50 µg of total RNA

*** based on average experimental recovery, results may vary**

Technical Support

For technical assistance, please call the **GenHunter Technical Support Hotline** at **800-311-8260** or **615-833-0665**.

You can also email a question or concern to us at: **info@genhunter.com**