



PRODUCT DATASHEET

Product Name: AffiEXTRACT® Total RNA Extraction Reagent

Catalog Number: AFG-KLE-009127

Package Size: 100 mL, 250 mL

Introduction

AffiEXTRACT® Total RNA Extraction Reagent is a reagent for total RNA extraction from various animal, plant, and bacterial cells and tissues. It has a strong cleavage ability to lyse cell and tissue samples in a short period of time while keeping the RNA intact. After centrifugation with chloroform, the solution forms a supernatant layer, an intermediate layer, and an organic layer (lower red layer). The RNA is distributed in the upper aqueous phase, and this supernatant layer is collected. Total RNA can be recovered by precipitation with propanol. The extracted total RNA is high in purity and contains no proteins or genomic DNA. It can be directly used in downstream applications such as Northern Blot, dot hybridization, mRNA purification, in vitro translation, RT-PCR, poly(A)+ selection, RNase protection analysis, construction of cDNA libraries, etc.

In addition, after removal of the aqueous layer, the DNA and proteins in the sample can also be successively reduced by precipitation and isolated. Ethanol precipitation can precipitate the DNA of the intermediate layer, and isopropanol can be added to the organic layer to precipitate the proteins. This product is fast and simple to operate. All operations can be completed in one hour. The AffiEXTRACT® Total RNA Extraction Reagent can perform well and lyse successfully both small amounts of tissue (50-100mg) and cells (5×10^6) and a large amounts of tissue ($\geq 1g$) and cells ($> 10^7$).

Notes

- Chloroform, isopropyl alcohol (for new or imported RNA), 75% ethanol (DEPC water), DEPC and DEPC water are not provided and should be self-supplied.
- Wear disposable clean gloves and operate in a separate clean area. Avoid talking during the operation to prevent contamination with RNases.
- Use RNase-free experimental equipment and consumables. The high temperature resistant material can be baked at 150°C for 4 hours to remove RNases, and other utensils can be soaked in 0.1% DEPC water overnight to remove RNases, and then sterilized and dried. Instruments and reagents for RNA experiments should be used exclusively and should not be used in other experiments.
- This product contains phenol, which is toxic and corrosive. If inhaled, in contact with skin, swallowed, etc., it can cause poisoning, burns and other bodily harm. Wear protective equipment such as protective clothing, gloves, eye masks, face masks, etc. when using this product. If your eyes accidentally come into contact with it, rinse immediately with plenty of water and seek medical help for treatment.
- After the sample is homogenized, if chloroform is not immediately added for downstream experiments, it can be frozen at -70 °C for more than one month. The RNA precipitate in 75% ethanol can be stored at 4°C for 1 week and stored at -20°C for 1 year.
- RNA's half-life is relatively short and it is easily degradable molecule. It is recommended to carry out subsequent experiments as soon as possible after extraction.

Sample size guidelines

In the below table we provide general guidelines on the volume/weight for the most common sample types that can be used per 1 mL of AffiEXTRACT® Total RNA Extraction Reagent.

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Sample type	Sample size
Adherent cells	10 cm ² Culture area
Suspended animal, plant, or yeast cells	5x10 ⁶ to 1x10 ⁷ cells
Bacterial cells	1x10 ⁷ cells
Whole blood	200 μ L
Animal tissue	30-100 mg
Plant tissue*	50-100 mg

*Plant tissues that contain low levels of polysaccharides and polyphenols should be used, e.g. young leaves, soft tissues, herbaceous plants. Plant tissues such as woody tissues, seeds, fruits, bark, mature leaves are often rich in polysaccharides (e.g., starch, pectin) and in polyphenols (e.g., tannins). Polysaccharides co-precipitate with proteins/DNA/RNA, and polyphenols bind irreversibly to proteins and nucleic acids. Both will influence negatively the purity of the isolated RNA and any potential downstream applications.

Note: The above-suggested volumes are merely indicative and the end user needs to determine the best ration for their specific samples. Please keep in mind that excessive sample volume can result in an insufficient lysis and a decrease in the purity of the isolated RNA.

Procedure

1. Sample preparation

1.1 Sample homogenization

A. Adherent cells

- Remove the culture medium completely. Add 1 mL of AffiEXTRACT® Total RNA Extraction Reagent directly to a 3.5 cm dish and immediately pipette up and down several times to lyse and homogenize the cells. Ensure the reagent fully covers the surface of the plate and all cells are detached. Incubate the lysate for 3-5 minutes at room temperature to allow complete dissociation of nucleoprotein complexes. Transfer the lysate to an RNase-free microcentrifuge tube.

B. Suspended cells

- Collect suspension cells by centrifugation at 300-500 \times g for 5 minutes. Carefully remove and discard the supernatant. Add 1 mL of AffiEXTRACT® Total RNA Extraction Reagent per 5x10⁶ to 1x10⁷ cells. Immediately pipette the mixture up and down repeatedly until the lysate is homogeneous and no visible cell clumps or granules remain. Incubate the lysate for 3-5 minutes at room temperature to allow complete dissociation of nucleoprotein complexes.
- Note: Do not wash the cells prior to lysis, as this may increase the risk of RNA degradation. For samples that are difficult to lyse (e.g., certain yeast or bacteria), use mechanical disruption methods such as a homogenizer.

C. Whole blood

Pipette 200 μ L of whole blood into an RNase-free tube. Add 1 mL of AffiEXTRACT® Total RNA Extraction Reagent and immediately mix thoroughly by pipetting up and down or gentle vortexing until the solution is homogeneous. Incubate the lysate for 3-5 minutes at room temperature to allow complete dissociation of nucleoprotein complexes.

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D. Animal / plant tissue

Option 1 - Frozen tissue (recommended for RNA integrity): Grind frozen tissue (stored at -70°C or below) thoroughly in liquid nitrogen to a fine powder. Add an appropriate volume of AffiEXTRACT® Total RNA Extraction Reagent according to the above table and immediately homogenize until the sample is uniform.

Option 2 - Fresh tissue: Mince fresh tissue into small pieces and add an appropriate volume of AffiEXTRACT® Total RNA Extraction Reagent according to the above table. Homogenize thoroughly using a suitable homogenizer until the lysate is uniform.

*Note: The sample volume should not exceed 10% of the AffiEXTRACT® Total RNA Extraction Reagent volume.

Mix the homogenate thoroughly (e.g., by vortexing) and incubate on ice for 5 minutes to promote complete dissociation of ribonucleoprotein complexes.

Clarification step (optional): Centrifuge at $12,000 \times g$ for 10 minutes at 4°C . Transfer the supernatant to a new RNase-free tube for further processing. Samples rich in protein, fat, or polysaccharides (e.g., adipose tissue, muscle, or plant storage organs) may require this step.

After centrifugation the pellet contains debris such as membranes, polysaccharides, and high-molecular-weight DNA and the supernatant contains RNA.

For adipose samples, remove the upper lipid layer as completely as possible and use only the clear lower phase for downstream processing.

2. Total RNA extraction

2.1 Add 300 μL of chloroform to the lysate. Cap the tube securely and shake vigorously for 15 seconds. Incubate at room temperature for 2–3 minutes.

2.2 Centrifuge at $12,000 \times g$ for 10–15 minutes at 4 – 10°C .

Following centrifugation, the mixture will separate into three distinct phases:

Upper aqueous phase (colorless): contains RNA

Interphase: contains DNA

Lower organic phase (red): contains proteins and phenol-chloroform

The aqueous phase typically represents ~ 50 – 60% of the initial AffiEXTRACT® Total RNA Extraction Reagent volume (e.g., ~ 500 – $600 \mu\text{L}$ when starting with 1 mL AffiEXTRACT® Total RNA Extraction Reagent).

2.3 Carefully transfer 400–500 μL of the upper aqueous phase to a new RNase-free tube. Avoid disturbing the interphase to prevent genomic DNA contamination.

2.4 Add an equal volume of isopropanol and mix thoroughly by gentle inversion. Incubate at -20°C for 20 minutes to precipitate RNA.

Note: RNA precipitation may not be visible prior to centrifugation. After centrifugation, a gel-like pellet may form at the bottom or along the side of the tube, especially when RNA yield is high.

2.5 Centrifuge at $12,000 \times g$ for 10 minutes at 4°C .

2.6 Carefully remove and discard the supernatant. Add 1 mL of 75% ethanol (prepared with RNase-free water) per 1 mL of AffiEXTRACT® Total RNA Extraction Reagent used initially. Gently resuspend the RNA pellet by flicking the tube or brief vortexing.

2.7 Centrifuge at $7,500$ – $12,000 \times g$ for 5 minutes at 4°C . Carefully discard the supernatant without disturbing the RNA pellet.

Note: If residual liquid remains, briefly centrifuge again and remove it with a pipette, taking care not to aspirate the pellet.

2.8 Air-dry the RNA pellet at room temperature for 2–5 minutes. Do not over-dry, as this may reduce RNA solubility.



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2.9 Dissolve the RNA pellet in 30–100 μL of RNase-free (DEPC-treated) water. Ensure complete dissolution before downstream use. Store RNA at -70°C or -80°C .

3. Product testing

3.1 RNA integrity

Mix 1 μL of RNA with an appropriate loading buffer and load onto a 1% agarose gel. Perform electrophoresis and visualize RNA bands.

Intact RNA typically shows a 28S rRNA band (~ 2 kb) and an 18S rRNA band (~ 1 kb). A clear band pattern indicates good RNA integrity.

Note: Band positions may vary depending on gel concentration and electrophoresis conditions.

3.2 RNA purity

Measure absorbance at 260 nm and 280 nm using a spectrophotometer. Calculate the A_{260}/A_{280} ratio. Pure RNA typically has a ratio of 1.9–2.2.