RNA Extraction Kit

Catalog No.: abx098089

Size: 50 rxns

Storage: Store the DNase I and DNase I Reaction Buffer at -20 °C for up to 12 months. Store the other kit components at room temperature (15-25 °C) for up to 12 months.

Introduction

Abbexa's RNA Extraction Kit is a fast and easy column-based method for the isolation of total RNA from cells and tissues. Cells and tissues are enzymatically lysed. DNA is digested with DNase I. RNA is bound to silica membrane. After washing, high quality RNA is eluted from the column. Isolated RNA is free of DNA and protein contamination, and is suitable for RT-PCR, qRT-PCR and Northern blot.

Kit components (Box 1 of 2)

1. Binding Buffer: 40 ml
2. Clean Buffer: 60 ml
3. Wash Buffer: 12 ml
4. Proteinase K Solution (20 mg/ml): 1 ml
5. RNase-Free Water: 10 ml
6. RNA Spin Columns with Collection Tubes: 50
7. RNase-Free Tubes: 50

Kit components (Box 2 of 2)

1. DNase I (3 units/µl)
2. DNase I Reaction Buffer: 4 × 1 ml

Material Required But Not Provided

1. 96-100% Ethanol
2. beta-Mercaptoethanol
3. Tryspin
4. Liquid Nitrogen
5. Lysozyme
6. TE Buffer
7. Pipettes and pipette tips
8. Centrifuge and centrifuge tubes
9. Homogenizer

Protocol

A. Reagent Preparation

- **Working Wash Buffer:** Dilute wash buffer 5-fold (1/5) with 96-100% ethanol before use (i.e. add 12 ml of wash buffer to 48 ml of ethanol). The total volume of the diluted wash buffer should be 60 ml.
- **Working Binding Buffer:** For each ml of Binding Buffer, add 10 µl of beta-Mercaptoethanol. The Working Binding Buffer solution should only be prepared prior to immediate use.
    - For animal cell number less than 1 × 10^6 cells, prepare at least 0.3 ml of Working Binding Buffer.
    - For animal cell number between 1 × 10^6 and 5 × 10^6 cells, prepare at least 0.6 ml of Working Binding Buffer.
    - For every 10 mg animal tissue, prepare at least 0.3 ml of Working Binding Buffer.
    - For bacterial cell number less than 1 × 10^9 cells, prepare at least 0.35 ml of Working Binding Buffer.
- **TE/Lysozyme Buffer**: (for bacterial cell samples only) Add 1 mg of lysozyme to 100 µl of TE buffer.
- **70% Ethanol**: (for animal cell and tissue samples only) Prepare a 70% ethanol solution using RNase-free water.
- **Working DNase I Solution**: For each 10 µl of DNase I (3 units/µl), add 70 µl of DNase I Reaction Buffer. If genomic DNA-free RNA is required, prepare at least 160 µl of Working DNase I solution.

B. Sample Preparation

- **Animal cells:** Adherent cells can be detached by trypsin or collected by removing the culture media. For suspension cells and detached adherent cells, transfer the cell suspension to a RNase-free tube and centrifuge at 12,000 × g for 5 minutes at 2-8 °C, and then discard the supernatant. Tap the tube gently to loosen the cell pellet. Add the appropriate volume of the Working Binding Buffer and then vortex at high speed to disperse cell pellet completely. Pipette up and down 5-10 times using a pipette with an RNase-free tip to homogenize the solution. Centrifuge at 12,000 × g for 5 minutes at room temperature then transfer the supernatant to a clean RNase-free tube.
- **Animal tissues:** Using liquid nitrogen, grind the sample and transfer the homogenate to a clean RNase-free tube. For every 10 mg of tissue, add 0.3 ml of Working Binding Buffer and 15 µl of Proteinase K and mix thoroughly by vortexing. Incubate for 10-20 minutes at 56 °C. Centrifuge at 12,000 × g for 5 minutes at room temperature then transfer the supernatant to a clean RNase-free tube.
- **Bacterial cells:** Centrifuge bacterial culture at 12,000 × g for 2 minutes at 2-8 °C to pellet bacterial cells. Discard the supernatant (If the supernatant is not completely removed, it may inhibit cell wall digestion in the next step). Add 100 µl of TE/Lysozyme Buffer to the pellet. Vortex at high speed to resuspend the pellet completely. Add 350 µl of Working Binding Buffer and mix thoroughly by vortexing. Allow to stand at room temperature for 5 minutes. Pipette up and down 5-10 times using a pipette with an RNase-free tip to homogenize the solution. Centrifuge at 12,000 × g for 5 minutes at room temperature then transfer the supernatant to a clean RNase-free tube.

Note:

- When using animal cell samples, the cell number should be ≤ 5 x 10^6.
- When using animal tissue samples, the weight of the tissue should be ≤ 20 mg.
- When using bacterial cell samples, the cell number should be ≤ 1 x 10^9.

C. Assay Procedure

1. **Animal cells and tissues:** Add equal volume of 70% ethanol to the lysate (use RNase-free Water to prepare the 70% ethanol).
   Bacterial cells: Add 250 µl of 96-100% ethanol to the lysate.
2. Vortex thoroughly to disperse any precipitate formed after adding ethanol. Centrifuge briefly and transfer the lysate into the spin columns. Centrifuge at 12,000 × g for 30 seconds, discard the flow-through. If the volume of lysate is larger than the capacity of the spin column, repeat this step.
3. Add 500 µl of Clean Buffer to the spin column. Centrifuge at 12,000 × g for 30 seconds. Discard the flow-through.
   Optional: If genomic DNA-free RNA is required, add 80 µl of Working DNase I solution and allow to stand for 15 minutes at room temperature.
4. Repeat step 3 once more.
5. Add 500 µl of Working Wash Buffer into the spin column. Centrifuge at 12,000 × g for 30 seconds at room temperature. Discard the flow-through.
6. Repeat step 5 once more.
7. Centrifuge the empty column at maximum speed (≥ 12,000 × g) for 2 minutes at room temperature in order to remove any ethanol residue present, then then air-dry the column matrix for several minutes.
8. Place the spin column into a clean 1.5 ml RNase free tube. Add 30-100 µl of RNase-free Water into the spin column matrix and allow to stand at room temperature for 1 minute.
9. Centrifuge at 12,000 × g for 2 minutes to elute the RNA.
10. Store the isolated RNA at -80 °C.