Code.:BR2B101

AcuGenix™ Brizol Reagent

V01

Product Description

AcuGenix™ Brizol Reagent is a broad-spectrum reagent for total RNA extraction. It is suitable for isolating total RNA from viruses, animal tissues, blood, plant materials, a wide range of microorganisms, and cultured cells. AcuGenix™ Brizol Reagent effectively inhibits RNase activity and enables efficient recovery of total RNA while minimizing contamination from DNA, proteins, and other impurities. The resulting RNA shows high integrity and can be used in routine molecular biology applications such as RT-PCR, RT-qPCR, Northern Blotting, Dot Blotting, In Vitro Translation, Poly(A) Selection, RNase Protection Assays, High-throughput Sequencing, and Molecular Cloning.

Components

Component	BR2B101 100 mL
AcuGenix™ Brizol Reagent	100 mL

Storage

Store at 2-8°C.

Materials

Isopropanol, chloroform, 75% ethanol, and RNase-free ddH_2O.

Notes

1. For Research Use Only. Not for use in diagnostic procedures.
2. AcuGenix™ Brizol Reagent contains toxic phenol. Avoid skin contact and inhalation. Wear protective goggles or use a transparent face shield to prevent splashing into the eyes. If skin contact occurs, rinse immediately with plenty of water. Seek medical attention promptly if discomfort persists.
3. Wear disposable gloves and a mask during operation to prevent RNase contamination.
4. All centrifuge tubes, pipette tips, and related tools must be free of RNase. Glassware can be baked at 150°C for 4 h to remove RNase. Plastic ware can be soaked in 0.5 M NaOH for 10 min, thoroughly rinsed with water, and then sterilized to remove RNase.
5. If RNA cannot be extracted immediately, samples homogenized in AcuGenix™ Brizol Reagent may be stored at -70°C.
6. If the RNA will be used for RT-PCR or RT-qPCR, even small amounts of genomic DNA may affect the experimental results. DNase I treatment can be performed in the downstream workflow if needed.
7. Total RNA extraction from frozen cells or tissues is usually less efficient than extraction from fresh samples because RNases may be released during freeze-thawing and can shear the RNA.
8. Use RNase-free ddH_2O when preparing solutions.

Protocol

1. Sample Processing

a. Plant tissues: Fresh plant tissue may be ground thoroughly in liquid nitrogen, or leaves may be cut into pieces and ground directly in AcuGenix™ Brizol Reagent. Perform grinding rapidly. Add 1 mL of AcuGenix™ Brizol Reagent per 15-50 mg of tissue.

b. Animal tissues: Cut the tissue into small pieces and place them in a glass homogenizer. Homogenize in 1 mL of AcuGenix™ Brizol Reagent per 15-50 mg of tissue. The sample volume should generally not exceed 10% of the AcuGenix™ Brizol Reagent volume. When high RNA integrity is required, freeze the tissue in liquid nitrogen, grind it in a chilled mortar, and then add AcuGenix™ Brizol Reagent for total RNA extraction.

c. Adherent cells: Centrifuge and collect the cells. Add 1 mL of AcuGenix™ Brizol Reagent per 10 cm^2 of cells. As a guide, use 1 mL per well of a 6 well plate and 0.5 mL per well of a 12 well plate. Gently rock 3-5 times and pipette up and down 2-3 times to ensure complete lysis, then transfer to a centrifuge tube. Alternatively, the cells may be treated with PBS containing 0.1%-0.25% trypsin. Once the cells detach, add serum-containing medium to inactivate the trypsin, transfer the cell suspension to an RNase-free centrifuge tube, centrifuge at 3,000 rpm at 4°C for 5 min, collect the cell pellet, and carefully remove all supernatant.

Note: Remove the culture medium completely before collecting the cells. Residual medium can cause incomplete lysis and reduce RNA yield.

d. Suspension cells: Centrifuge and collect the cells. Add 1 mL of AcuGenix™ Brizol Reagent to 5×10^6-1×10^7 animal or plant cells, yeast cells, or bacteria. Pipette up and down to ensure complete lysis. For yeast or bacterial samples that are difficult to lyse, use a homogenizer if needed to ensure complete disruption.

Note: Do not wash the cells before adding AcuGenix™ Brizol Reagent, as this may lead to mRNA degradation.
e. Blood and viral samples: Add 3 volumes of AcuGenix™ Brizol Reagent directly to fresh whole blood or viral suspension. A typical recommendation is 0.6 mL of AcuGenix™ Brizol Reagent for 0.2 mL of whole blood or viral sample.
2. After adding an appropriate volume of AcuGenix™ Brizol Reagent according to the sample type, incubate at room temperature for 5 min to ensure complete lysis.
3. Optional step: Centrifuge at 12,000 rpm at 4°C for 10 min and transfer the supernatant to a new tube.
Note: If the sample contains large amounts of protein, fat, polysaccharides, muscle tissue, or plant nodules, this centrifugation step helps remove insoluble material. The pellet contains extracellular membranes, polysaccharides, and high-molecular-weight DNA, whereas the supernatant contains RNA. When processing fatty tissues, remove the upper lipid layer and use the clear homogenate for the next step.
4. Add 0.2 mL of chloroform per 1 mL of AcuGenix™ Brizol Reagent. Mix thoroughly by pipetting and incubate at room temperature for 2-3 min.
Note: The sample may also be mixed by vortexing or by vigorous manual inversion for 15 s.
5. Centrifuge at 12,000 rpm at 4°C for 15 min. The mixture will separate into three layers: a red organic phase, an interphase, and a colorless upper aqueous phase. Transfer the colorless upper aqueous phase containing total RNA to a new centrifuge tube.
6. Add an equal volume of isopropanol to the recovered aqueous phase and mix by gentle inversion several times. Allow the RNA to precipitate at room temperature for 10 min. For recovery of small RNAs such as microRNA, overnight precipitation at -70°C is recommended.
7. Centrifuge at 12,000 rpm at 4°C for 10 min. An RNA pellet should be visible at the bottom of the tube. Discard the supernatant.
8. Add 1 mL of 75% ethanol prepared with RNase-free ddH_2O and mix by gentle inversion.
9. Centrifuge at 12,000 rpm at 4°C for 5 min and discard the supernatant. Do not dislodge the pellet. Briefly centrifuge again to collect residual liquid and remove it carefully with a pipette.
10. Air-dry at room temperature for 2-3 min. Add 30-100 µL of RNase-free ddH_2O as required, pipette repeatedly to dissolve the RNA completely, and store at -70°C. Do not overdry the RNA pellet, as excessively dried RNA is difficult to dissolve and may give an A_{260}/A_{280} value below 1.6.

FAQs

1. Low yield
a. Sample lysis or homogenization was incomplete.
b. The RNA pellet was not completely dissolved.
2. $A_{260}/A_{280} < 1.65$
a. The RNA sample was dissolved in water rather than TE buffer before absorbance measurement.
b. Too little AcuGenix™ Brizol Reagent was added during homogenization.
c. The homogenized sample was not left at room temperature for 5 min.
d. Organic phase contamination was carried over into the aqueous phase.
e. The final RNA pellet was not fully dissolved.
3. RNA degradation
a. Tissue samples were not processed or frozen immediately after collection.
b. The sample or extracted RNA pellet was not stored at -70°C.
c. Cells were damaged during trypsin treatment.
d. The solutions or centrifuge tubes were not treated to remove RNase.
4. DNA contamination
a. Too little AcuGenix™ Brizol Reagent was added during homogenization.
b. The sample contained tissue solvents such as ethanol or DMSO, or contained a strong buffer or alkaline solution.
5. Protein/polysaccharide contamination
a. Excessive protein or polysaccharide content. b. Sample input too large; use more AcuGenix™ Brizol Reagent. c. Organic phase carried into the aqueous phase.