CYRN70-CHANGYU AIDpure Total RNA Kit (Chloroform-free)

Catalog number	Package
CYRN7001	50 preps
CYRN7002	100 preps

Storage and shipping: 15-25 °C, except AIDzol Reagent (4 °C protected from light). Stable for 12 months. AIDzol Reagent can be shipped at room temperature, stored long-term at 4 °C in the dark, and kept at room temperature for 3 months without loss of quality. Keep all solutions tightly capped after use.

Kit components

Component	50 preps (CYRN7001)	100 preps (CYRN7002)
AIDzol Reagent	25 ml (4 °C, dark)	50 ml (4 °C, dark)
Deproteinization Buffer PE	16 ml	32 ml (add ethanol before first use)
Wash Buffer RW	10 ml	25 ml (add ethanol before first use)
RNase-free H2O	5 ml	10 ml
RNase-free spin columns RA	50	100
Collection tubes (2 ml)	50	100

Description

AIDzol is a chloroform-free upgrade of conventional Trizol and is widely suitable for total RNA extraction from animal tissues, plant materials, cultured cells and bacteria. Compared with conventional Trizol, it does not require chloroform phase separation, is simpler to use and can be performed entirely at room temperature; AIDzol also removes DNA more effectively than conventional Trizol, so the resulting RNA is free of DNA contamination and can be used directly in downstream experiments without DNase digestion. The kit includes spin-column technology; repeated washes remove impurities, simplify the procedure and maximize RNA purity.

Features

The silica membrane is specially selected; variation in binding capacity between columns is minimal and reproducibility is high.

Combines the stability and purity of the guanidinium thiocyanate/phenol one-step reagent with the convenience of a spin column. No isopropanol precipitation or ethanol washing is required, and RNA is eluted directly from the column, avoiding the poor solubility of over-dried pellets.

The dedicated AIDzol formulation effectively eliminates residual genomic DNA.

Repeated wash steps remove protein and give higher RNA purity.

Effectively reduces the 5S rRNA content of total RNA and improves purity.

Notes before starting

Note: The kit inhibits RNases very effectively; unless otherwise stated, all centrifugation steps can be performed at room temperature.

Note: AIDzol Reagent and Deproteinization Buffer PE contain irritant compounds. Wear gloves and avoid contact with skin, eyes and clothing. Rinse affected areas with plenty of water or saline.

Note: Conventional agarose gel electrophoresis and formamide-denaturing gel electrophoresis can both be used to assess RNA quality. Good RNA shows two prominent rRNA bands after denaturing electrophoresis at about 5 kb (28S) and 2 kb (18S) (on ordinary agarose gels 28S and 18S appear at about 2 kb and 1 kb, and their positions vary with RNA secondary structure), with a brightness ratio of about 2:1. Bands at about 0.1 kb and 0.3 kb (5S and tRNA) may also be visible. Four or five bands can be normal in some species or plant tissues, and discontinuous high-molecular-weight bands between 7 kb and 15 kb may appear if RNA precursors, heterogeneous nuclear RNA or small nuclear RNA are extracted.

Note: After homogenization in AIDzol and before adding water and centrifugation, the sample can be stored at -60 to -70 °C for more than one month.

Note: For bacterial RNA, the EASYspin bacterial RNA kit (CYRN43) is recommended.

❖ Procedure

Before first use, add absolute ethanol to Wash Buffer RW and Deproteinization Buffer PE as indicated on the labels, mix well and tick the boxes to avoid adding ethanol twice.

1. Sample processing. a. Plant tissue: grind fresh plant tissue thoroughly in liquid nitrogen, or cut it into small pieces and grind directly in AIDzol; add 0.5 ml of AIDzol per 25-50 mg of tissue and mix. b. Animal tissue: cut fresh or -70 °C-frozen tissue into small pieces and add 0.5 ml of AIDzol per 15-50 mg, then homogenize; or grind in liquid nitrogen and add 0.5 ml of AIDzol and mix. c. Monolayer cultured cells: remove residual medium completely, add 0.5 ml of AIDzol directly to a 3.5 cm dish, cover and pipette repeatedly to lyse the cells. Choose the AIDzol volume by plate area, not cell number (0.5 ml per 10 cm^2); insufficient AIDzol can cause DNA contamination in the RNA. Note: adherent cells often do not detach completely from the flask or dish, which does not mean incomplete lysis; the membrane has in fact ruptured and released all RNA, so continue. d. Cell suspension: collect cells by centrifugation and lyse by pipetting repeatedly in AIDzol. Add 0.5 ml of AIDzol per 1-5×10^6 animal or plant cells, or per 5×10^6 bacteria; avoid washing cells before adding AIDzol, since this increases the risk of mRNA degradation. Some bacteria may require homogenization. e. Liquid samples: for each 200 μl of plasma, serum or other liquid sample (adjust to 200 μl with deionized or purified water if less), add 0.5 ml of AIDzol and vortex.

2. Add 2/5 volume of deionized or purified water to the lysate (ordinary laboratory water is sufficient; it need not be RNase-free; 200 μl of water per 500 μl of AIDzol), vortex vigorously and leave at room temperature for 5 minutes. For larger samples of about 50 mg, the room-temperature incubation can be extended to 10-15 minutes.

3. Centrifuge at room temperature at 12,000 rpm for 15 minutes.

4. After centrifugation the solution separates into an upper aqueous phase containing RNA and a lower pellet containing protein, DNA, polysaccharides and other impurities. Carefully transfer the upper aqueous phase to a new centrifuge tube. The upper aqueous phase is about 90% of the total volume; for 500 μl of AIDzol the upper phase is about 630 μl, and 500 μl is recommended. For micro samples, transfer all of the supernatant to reduce RNA loss. For small samples, no lower pellet may be visible after centrifugation; this is normal, and the procedure can be continued.

5. Add 0.5 volume (half the aqueous volume) of absolute ethanol and mix (a precipitate may form). Transfer the solution, together with any precipitate, to spin column RA placed in a collection tube and centrifuge at 12,000 rpm for 30 seconds. Discard the flow-through and return the column to the collection tube. The column capacity is 750 μl; if the mixture exceeds this volume, load it in several portions, discarding the flow-through and returning the column to the collection tube between loads.

6. Add 500 µl of Deproteinization Buffer PE (check that ethanol has been added), centrifuge at 12,000 rpm for 30 seconds and discard the flow-through.

7. Add 500 µl of Wash Buffer RW (check that ethanol has been added), centrifuge at 12,000 rpm for 15 seconds and discard the flow-through.

8. Repeat step 7 once.

9. Return column RA to the empty collection tube and centrifuge at 12,000 rpm for 2 minutes to remove residual wash buffer, since residual ethanol can inhibit downstream reactions.

10. Transfer column RA to a new 1.5 ml centrifuge tube. Apply 40-60 µl of RNase-free ddH2O to the centre of the membrane, leave for 2 minutes and centrifuge at 12,000 rpm for 1 minute.

Use an elution volume of at least 30 µl; smaller volumes reduce recovery. To increase RNA concentration, prewarm the RNase-free ddH2O to 90 °C before elution or reload the first eluate onto the column for a second elution.

This product is furnished for LABORATORY RESEARCH USE ONLY. Not for diagnostic or therapeutic use.