PureZol Reagent

Cat. No. ABTGNA201-100

Storage: at 4°C in dark for one year

Description

PureZol Reagent is a ready-to-use reagent for the isolation of total RNA from cells and tissues. PureZol Reagent combines phenol and guanidine thiocyanate in a mono-phase solution to inhibit RNase. After lysis and centrifugation, RNA remains in the aqueous phase and others in the interphase or organic phase. RNA is precipitated by addition of isopropanol.

- Isolate RNA from a variety of species: animal, plant, yeast, bacteria and virus.
- The whole procedure can be completed in one hour.
- Simultaneous isolation of RNA, DNA and protein from the same sample.
- Pink solution for easy visualizing different phases.
- Unique dissolving solution for long-term RNA storage.

Procedures

Reagents provided by customers: chloroform, isopropanol, 75% ethanol (prepared with RNase-free water) and RNase-free water

1. Homogenization

a. Adherent cells

- Wash culture dish once with 1×PBS
- Detach cells with cell spatula. Add 1 ml of PureZol Reagent to per 10 cm^3 culture dish. Pipetting up and down to lysis the cells.
- Transfer lysate to a microcentrifuge tube.
- Incubate at room temperature for 5 minutes.

b. Suspension cells

- Transfer suspension cells to a microcentrifuge tube. Centrifuge the sample at 8,000× g for 2 minutes at 4°C, discard the supernatant.
- Add 1 ml of PureZol Reagent to per 10^7 cells.
- Pipetting up and down until no visible precipitates are present in lysate.
- Incubate at room temperature for 5 minutes.

c. Animal tissue and plant materials

- After weighing, quickly transfer the frozen sample into mortar with liquid nitrogen. Grind thoroughly to a powder. Use more liquid nitrogen if needed. Incomplete grind can affect RNA yield and quality.
- Transfer the tissue powder to a microcentrifuge tube. Add 1 ml of PureZol Reagent to per 50-100 mg tissue. Homogenize tissue samples with a homogenizer and repetitively pipette up and down.
- Incubate at room temperature for 5 minutes.

2. Add 0.2 ml of chloroform for per ml PureZol Reagent used. Shake the tube vigorously by hand for 30 seconds. Incubate at room temperature for 3 minutes.

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3. Centrifuge the sample at 10,000×g for 15 minutes at 4°C. The mixture separates into a lower pink organic phase, an interphase, and a colorless upper aqueous phase which contains the RNA. The volume of the aqueous upper phase is around 60% volume of PureZol Reagent .
4. PureZol Reagent the colorless, upper phase containing the RNA to a fresh RNase-free tube. Add 0.5 ml of isopropanol for per ml PureZol Reagent used. Mix thoroughly by inverting tube. Incubate at room temperature for 10 minutes.
5. Centrifuge the sample at 10,000×g for 10 minutes at 4°C. Discard the supernatant. Colloidal precipitate can be seen at the wall and the bottom of the tube.
6. Add 1 ml of 75% ethanol (prepared with RNase-free water), vortexing vigorously (add at least 1 ml of 75% ethanol for 1 ml PureZol Reagent used).
7. Centrifuge the sample at 7,500×g for 5 minutes at 4°C.
8. Discard the supernatant. Air-dry the RNA pellet (about 5 minutes).

RNA resuspension

1. Resuspend the RNA pellet in RNase-free water or 0.5% SDS solution (20–50 µL) by passing the solution up and down several times through a pipette tip.
   Note: Do not dissolve the RNA in 0.5% SDS if it is to be used in subsequent enzymatic reactions.
2. Incubate in a water bath or heat block set at 55–60°C for 10–15 minutes.
3. Proceed to downstream application, or store at –70°C.

Note

It is important to mix well after adding chloroform to ensure extraction performance.