Total RNA Extraction

Material

RNAiso Plus(Takara Co.9109)

Procedure

1. Collection
    1) Collect and pipettes 1.5~2.5ml bacteria which is in the log phage(usually when OD600=1.0) into a centrifuge tube. Centrifuge tube for 8,000×g,5 minutes at 4℃.Discard supernatant and be care not to disturb the bacteria pellet.
    2) Add 1ml of RNAiso Plus, pipette up and down until pellet is completely resuspended.
    3) Leave at room temperature(15~30℃) for 5 minutes, isolate the RNA from the nuclear protein.
2. Extracion of total RNA
    1) Add 200 ul chloroform, cap the centrifuge tube and mix until the solution becomes milky.
    2) Keep the solution at room temperature for 5 minutes.
    3) Centrifuge at 12,000×g for 15 minutes at 4℃.Centrifuging the solution will separate it into three layers; liquid top layer(contains RNA),semisolid middle layer(mostly DNA),and bottom organic solvent layer.
    4) Transfer the top liquid layer to new centrifuge tube without touching middle layer.
    5) Measure the amount of the top layer and add an equal amount or add up to 0.5 times of isopropanol of the top layer. Mix together well. Keep the mixture at room temperature for 10 minutes.
    6) Centrifuge at 12,000×g for 10 minutes at 4℃ to precipitate the RNA.
    7) Cleaning RNA precipitate
    8) Carefully remove the supernatant, do not touch the pellet.
    9) Add an amount of 75% cold ethanol that was equivalent to the supernatant. Clean the precipitate by vortexing.
    10) Centrifuge the solution at 7,500×g for 5 minutes at 4℃ and discard supernatant. Be care not to disturb the precipitate.
3. Dissolving RNA

    Dry the precipitate by leaving the tube open for several minutes. After the precipitate is dry, dissolved it with appropriate amount of RNase-free water.

Attention

Make sure that all the centrifuge tubes and pipettes have been treated with DEPC.