(DP424) TRNzol Universal Reagent ——Animal Tissues

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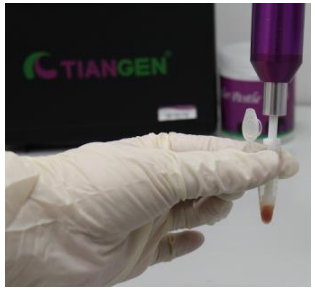
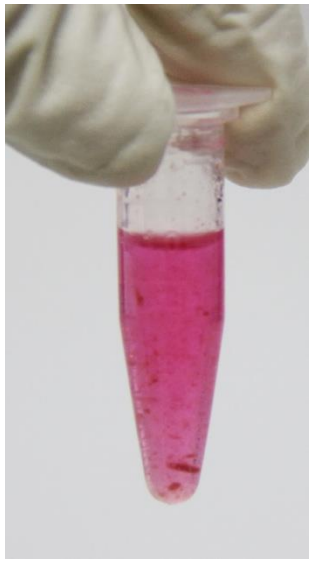
Experiment Preparation

1. Animal tissue; Mortar; Liquid nitrogen
2. Chloroform; Isopropanol; RNase-Free ddH_2O; 75% ethanol (Prepare with RNase-Free ddH_2O)
3. Pipette and matched RNase-free sterile tips (200 μl, 1 ml); 1.5 ml centrifuge tubes (RNase-free)
4. Vortex oscillator; Refrigerated centrifuge



Step 1

Add less than 150 μl TRNzol Universal in tissue sample, homogenize until there's no obvious clumps, or fully grind in the mortar with liquid nitrogen.



Refill TRNzol Universal per 50–100 mg tissue to 1 ml and mix well quickly.

Note: the sample volume should not exceed one-tenth of the volume of TRNzol Universal (1 ml).

Step 2

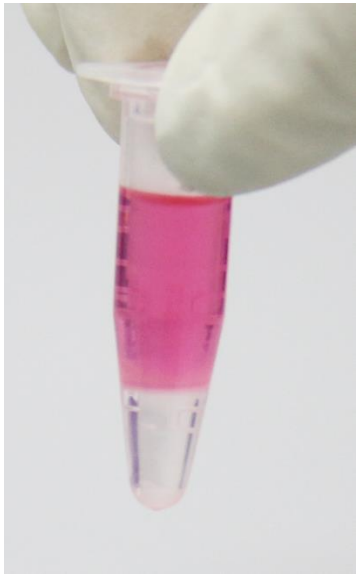
Place the homogenate samples in 15-30℃ for 5 min to make the nucleic acid protein complex completely separate. The color of the sample will become slightly darker at this step.

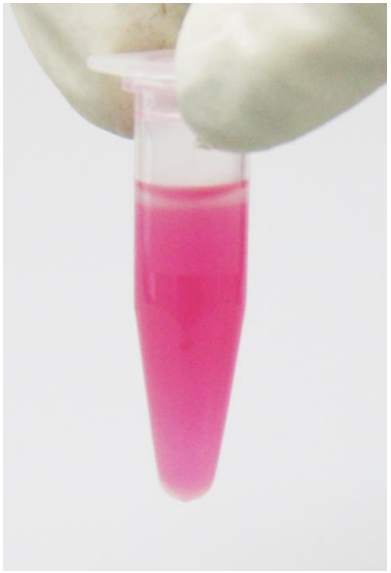
Step 3 (Optional)

Centrifuge at 12,000 rpm (~13,400 g) at 4℃ for 5 min, transfer the supernatant into a new RNase-free centrifuge tube.

Note: If the sample contains more protein, fat, polysaccharide or muscle parts, etc., this step can be included. The precipitates obtained by centrifugation include extracellular membrane, polysaccharide, while high molecular weight DNA and RNA in the supernatant solution.

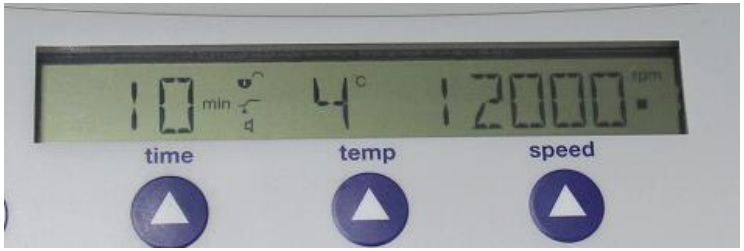
Step 4

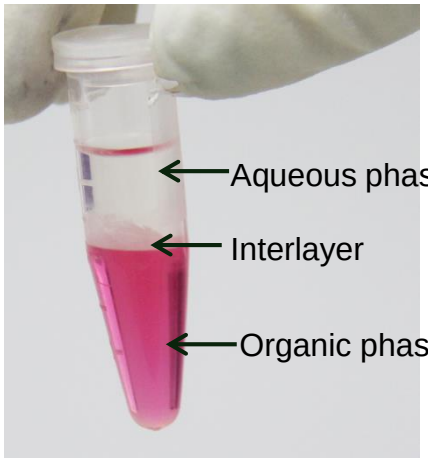




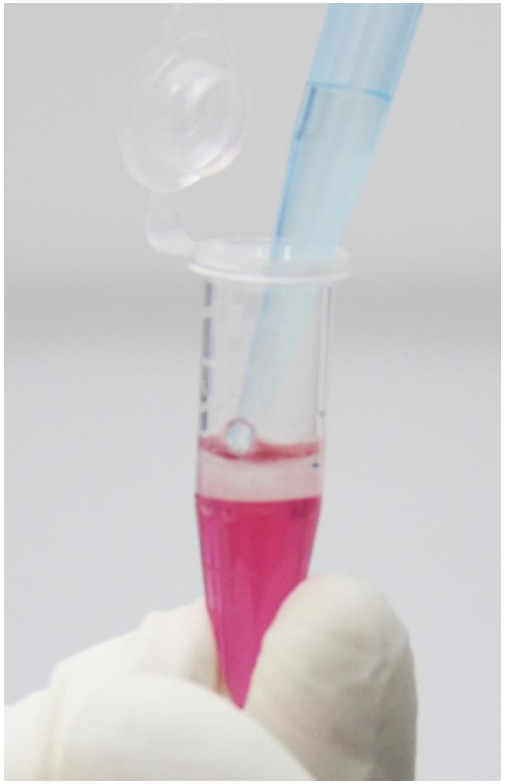
Add 200 μl chloroform and vortex vigorously for 15 sec.
Then place at room temperature for 3 min.

Step 5





Centrifuge at 12,000 rpm (~13,400×g) at 4℃ for 10 min.

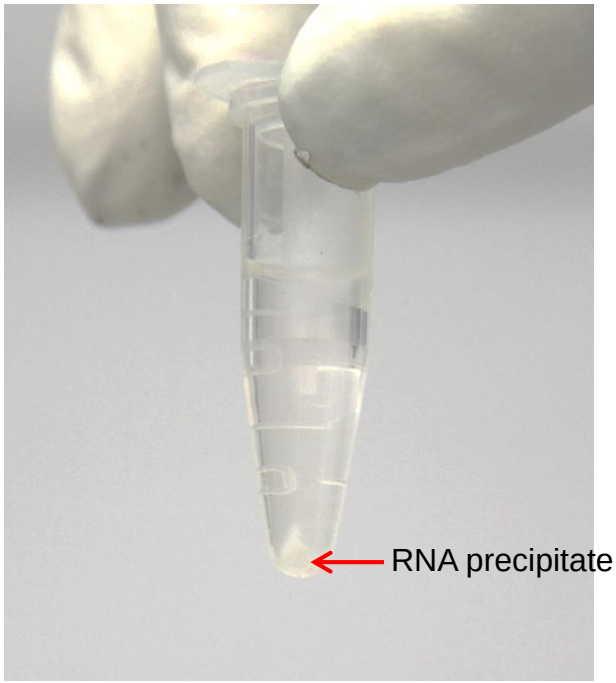


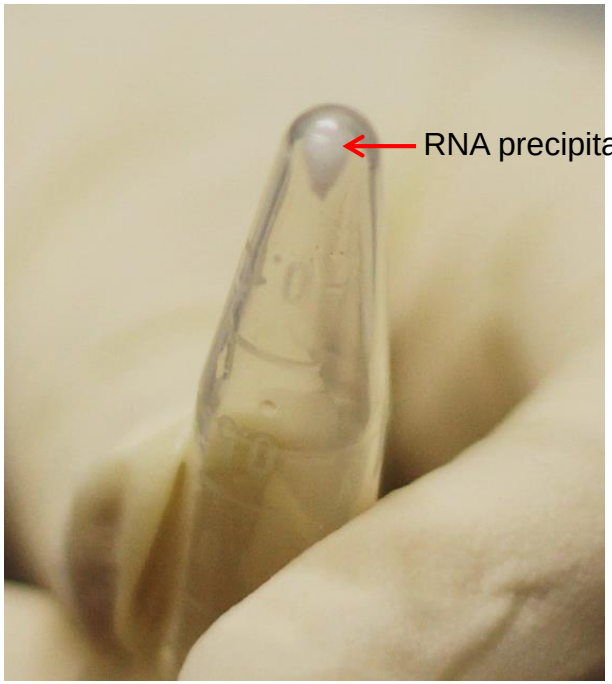
Transfer the water phase to a new tube for the next step. The volume of the water phase is approximately 50% of that of the TRNzol Universal used.

Step 6

Add the same volume of isopropanol in the obtained water solution, mix well and place at room temperature for 10 min.

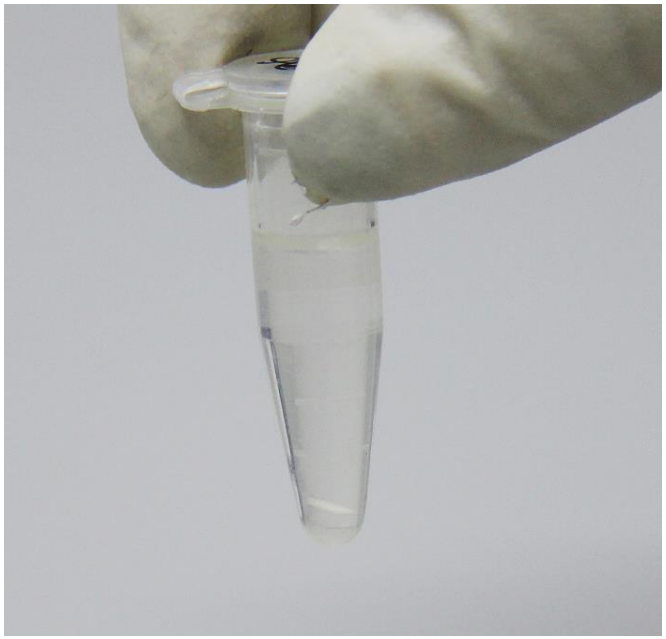
Step 7





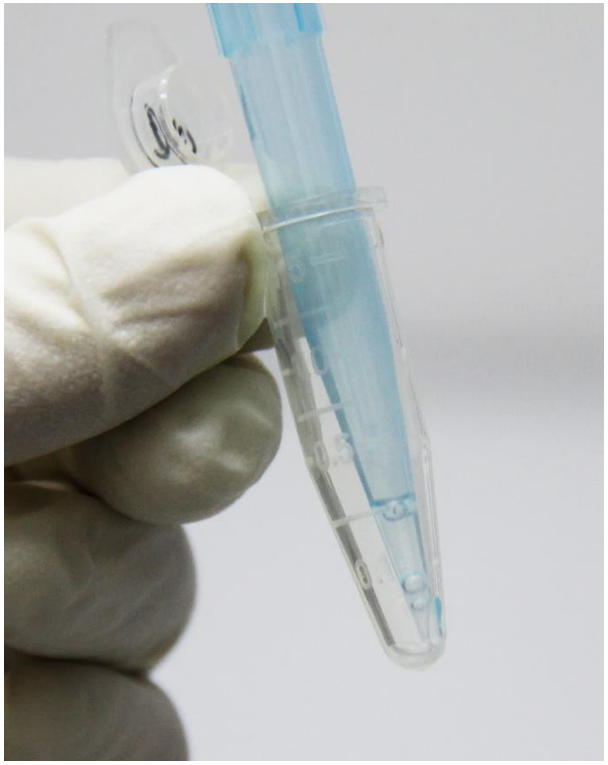
Centrifuge at 12,000 rpm (~13,400×g) at 4℃ for 10 min, and remove the supernatant. RNA precipitation is often invisible before centrifugation, and colloidal precipitation will be formed on the side and bottom of the tube after centrifugation.

Step 8



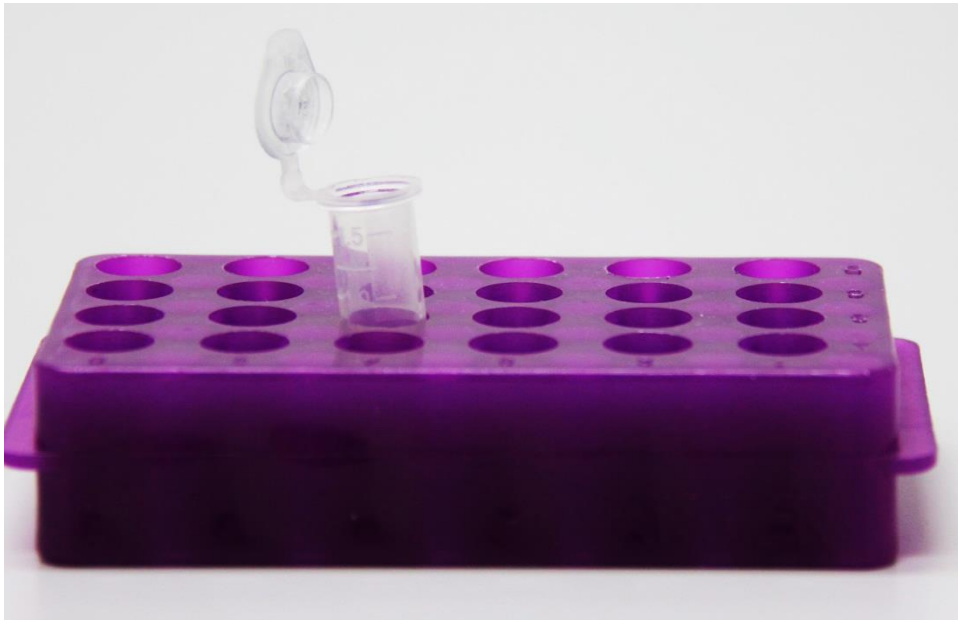
Add 1 ml 75% ethanol (prepare with RNase-free ddH_2O) to wash the precipitation. Wash with at least 1 ml 75% ethanol for every 1 ml TRNzol Universal.

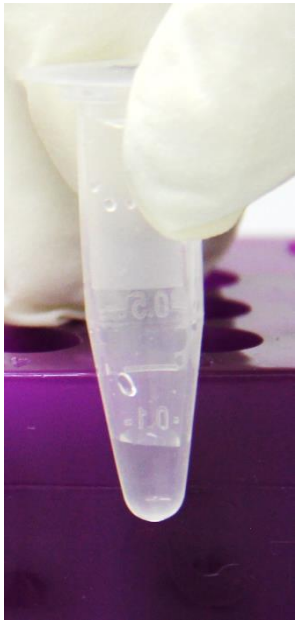
Step 9



Centrifuge at 10,000 rpm (~9,391×g) at 4℃ for 5 min, and discard the supernatant. Be careful not to pour out the precipitate. Centrifuge the remaining small amount of liquid for a short time, and then carefully remove the liquid without touching the precipitate.

Step 10





Dry at room temperature for 2-3 min (Do not over-dry, or the RNA will be difficult to dissolve). According to the experiment needs, add 30-100 μl RNase-free ddH_2O, pipette up and down repeatedly to fully dissolve RNA.