RNA Extraction from Duck Spleen Tissues using TRIzol Reagent

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Abstract

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Protocol

Add 1 mL of TRIzol™ Reagent per 50–100 mg of tissue to the sample and homogenize using a homogenizer.

Step 1.

NOTES

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TRIzol Reagent by Thermo Fisher Scientific Catalog #:15596026

Incubate for 5 minutes to permit complete dissociation of the nucleoproteins complex.

Step 2.

Add 0.2 mL of chloroform per 1 mL of TRIzol™ Reagent used for lysis, then securely cap the tube.

Step 3.

Incubate for 2–3 minutes.

Step 4.

Centrifuge the sample for 15 minutes at 12,000 × g at 4°C.

Step 5.

Transfer the aqueous phase containing the RNA to a new tube.

Step 6.

Transfer the aqueous phase containing the RNA to a new tube by angling the tube at 45°and pipetting the solution out.

Step 7.

Add 0.5 mL of isopropanol to the aqueous phase, per 1 mL of TRIzol™ Reagent used for lysis.

Step 8.

Incubate for 10 minutes.

Step 9.

Centrifuge for 10 minutes at 12,000 × g at 4℃.

Step 10.

Discard the supernatant with a micropipettor.

Step 11.

Resuspend the pellet in 1 mL of 75% ethanol per 1mL of TRIzolTMReagent used for lysis.

Step 12.

Vortex the sample briefly, then centrifuge for 5 minutes at 7500×g at 4℃.

Step 13.

Discard the supernatant with a micropipettor.

Step 14.

Air dry the RNA pellet for 5-10 minutes.

Step 15.

Resuspend the pellet in 20-50 µL of RNase-free water solution by pipetting up and down.

Step 16.

Incubate in a water bath or heat block set at 55-60℃ for 10-15 minutes.

Step 17.

Proceed to downstream applications, such as DNase treatment or cDNA synthesis, or store at -70˚C.

Step 18.