Lysate Preparation from Viruses in Mammalian Tissue

Bioline

Abstract

The steps for preparing the lysate are different depending on the starting material. Please ensure you follow the correct procedure for your starting material (see the section Disrupting and Homogenizing Starting Materials and the different lysate preparation protocols here). The subsequent steps detailed in Genomic DNA removal and total RNA purification are the same in all cases.

This protocol describes the isolation of viral RNA from small amounts of tissue (up to 20 mg for most types of tissue).

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Guidelines

- This protocol describes the isolation of viral RNA from small amounts of tissue (up to 20 mg for most types of tissue). Refer to Table 1 as a guideline for maximum tissue input amounts. If your tissue of interest is not included in the table, we recommend starting with an input of no more than 10 mg.

TABLE 1: RECOMMENDED INPUT OF DIFFERENT TISSUES

Tissue	Max. Input Amount
Brain	25 mg
Heart	5 mg
Kidney, Liver, Lung, Spleen	20 mg
Other tissues	10 mg

- RNA in mammalian tissue is not protected from RNases after harvesting until the tissue is disrupted and homogenized. Therefore, it is important that the procedure is carried out as quickly as possible.

- Fresh or frozen tissues may be used for the procedure. Tissues should be flash-frozen in liquid nitrogen and transferred immediately to a -80°C freezer for long-term storage. Tissues may be stored at -80°C for several months. Do not allow frozen tissues to thaw prior to grinding with the mortar and pestle in order to avoid compromising the integrity of the RNA.

Please review the Guidelines under Genomic DNA removal and total RNA purification from all types of lysate for other important details.

Before start

- Ensure Lysis Buffer RX is prepared (see guidelines).
- Ensure that all solutions are at room temperature before use.
- Two types of spin columns are provided with this kit: the **Genomic DNA Removal Column** (blue ring) and the **RNA Column** (black ring). Ensure the correct column is used for each step of the procedure.
- All centrifugation steps are carried out in a benchtop microcentrifuge at 14,000 x g except where noted. Perform all centrifugation steps at room temperature.
- It is important to work quickly during this procedure.

Materials

ISOLATE II Biofluids RNA Kit BIO-52086 by Bioline

Protocol

Step 1.
Determine amount of tissue by weighing.

NOTES
Steve Hawkins 08 Dec 2016

The steps for preparing the lysate are different depending on the starting material. Please ensure you follow the correct procedure for your starting material (see the different lysate protocols here). The subsequent steps (detailed here) for Genomic DNA Removal and Total RNA Purification are the same in all cases.

Step 2.

Transfer the tissue into a mortar that contains enough liquid nitrogen to cover the

sample. Grind thoroughly using a pestle.

Step 3.
Allow the liquid nitrogen to evaporate, without allowing the tissue to thaw.

Step 4.

Add 600 µL of Lysis Buffer RX to the tissue sample and continue to grind until the

sample has been homogenized.

Step 5.

Homogenize by passing the lysate through a nuclease-free 20 gauge (0.9 mm) needle attached to a syringe 5-10 times.

Step 6.

Using a pipette, transfer the lysate into a 1.5 mL RNase-free microcentrifuge tube (user supplied).

Step 7.
Spin the lysate for 2 min to pellet any cell debris.

DURATION
00:02:00

Step 8.

Transfer the supernatant to another 1.5 mL RNase-free microcentrifuge tube. Note the volume of the supernatant/lysate.

Step 9.

Proceed to Genomic DNA removal and total RNA purification from all types of lysate.

Warnings

When working with chemicals, always wear a suitable lab coat, gloves and safety glasses.

Lysis Buffer RX contains guanidinium thiocyanate. This chemical is harmful in liquid form when in contact with skin or ingested. If the solution is allowed to dry, the powder is harmful if inhaled.

CAUTION: Do not add bleach directly to solutions or sample preparation waste containing guanidinium salts. Reactive compounds and toxic gases can form. In the case of spillage, clean the affected area with a suitable laboratory detergent and water.

For detailed information, please consult the material data safety sheet (MSDS) available on our website at www.bioline.com.

Biofluids derived from all human and animal sources are considered potentially infectious. All necessary precautions recommended by the appropriate authorities in the country of use should be taken when working with biofluids.