Lysate Preparation from Viruses in Cultured Cells Growing in Suspension and Lifted Cells

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 is for the isolation of integrated viral RNA from mammalian cells growing in culture.

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Guidelines

- A maximum of 3 x 10^6 eukaryotic cells can be used as starting material. A hemocytometer can be used in conjunction with a microscope to count the number of cells. As a general guideline, a confluent 3.5 cm plate of HeLa cells will contain 10^6 cells.
- Cell pellets can be stored at -80°C for later use or used directly in the procedure. Determine the number of cells present before freezing.
- Frozen pellets should be stored for no longer than 2 weeks to ensure that the integrity of the RNA is not compromised.
- Frozen cell pellets should not be thawed prior to beginning the protocol. Add the Lysis Buffer RX directly to the frozen cell pellet.

Additional reagents required: PBS (RNase-free).

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.

Transfer cell suspension to a 1.5 mL RNase-free microcentrifuge tube (user supplied).

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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.

Centrifuge at no more than 200 x g for 10 min to pellet cells.

DURATION
00:10:00

Step 3.
Carefully decant the supernatant to ensure that the pellet is not dislodged. Ensure that a few microliters of media are left behind with the pellet in order to ensure that the pellet is not dislodged.

Step 4.
Add 350 µL of Lysis Buffer RX to the pellet. Lyse cells by vortexing for 15s. Ensure entire pellet is completely dissolved before proceeding to the next step.

DURATION
00:00:15

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Note: For input amounts greater than 10^6 cells, it is recommended that the lysate is passed through a nuclease-free 20 gauge (0.9 mm) syringe needle 5-10 times, in order to reduce the viscosity of the lysate prior to loading onto the column.

Step 5.

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.