Lysate Preparation from Viruses in Suspension

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 <u>Disrupting and Homogenizing Starting Materials</u> and the different lysate preparation protocols <u>here</u>). The subsequent steps detailed in <u>Genomic DNA removal and total RNA purification</u> are the same in all cases.

Follow this protocol for isolating RNA from free viral particles.

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Guidelines

It is recommended that no more than 100 μ L of viral suspension be used in order to prevent clogging of the column.

Please review the Guidelines under <u>Genomic DNA removal and total RNA purification from all types of lysate</u> 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 up to 100 µL of viral suspension to a 1.5 mL RNase-free microcentrifuge tube (user supplied).

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.

Add 350 µL of Lysis Buffer RX.

NOTES

Steve Hawkins 18 Oct 2016

Note: This volume of lysis buffer can be used for a culture plate of \sim 35 mm in diameter or up to one well of a 6-well culture dish.

Step 3.

Lyse cells by vortexing for 15s. Ensure that mixture becomes transparent before proceeding with the protocol.

O DURATION

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Step 4.

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.