Lysate Preparation from Biofluids/Viruses Collected with Nasal or Throat Swabs

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 designed for the isolation of all sizes of RNA (including miRNA) from a wide variety of biofluids derived from all human and animal sources collected with nasal or throat swabs.
- Follow this protocol for isolating RNA from viruses collected with nasal or throat swabs.

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Guidelines

Please review the Guidelines under Genomic DNA removal and total RNA purification from all types of lysate for 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 Colum** (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.
Add 600 µL of Lysis Buffer RX 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.
Gently brush a sterile, single-use cotton swab inside the nose or mouth.

Step 3.

Using sterile techniques, cut the shaft of the cotton tip and place the tip containing the collected cells into the microcentrifuge tube containing Lysis Buffer RX.

DURATION
00:00:15

Step 4.

Close tube and vortex gently. Incubate for 5 min at room temperature.

DURATION
00:05:00

Step 5.

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

Step 6.

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.