SECTION 1. LYSATE PROTOCOLS

1.1 PREPARATION FROM BIOFLUIDS / VIRUSES

1.1.1 Protocol

1. Transfer up to 100µl of biofluid to a 1.5ml RNase-free microcentrifuge tube (user supplied).
2. Add 350µl of Lysis Buffer RX to the sample. Lyse cells by vortexing for 15s. Ensure that mixture becomes transparent before proceeding with the protocol. **Proceed to section 2.**

1.2 LYSATE PREPARATION FROM BIOFLUIDS / VIRUSES COLLECTED WITH NASAL OR THROAT SWABS

1.2.1 Protocol

1. Add 600µl of Lysis Buffer RX to a 1.5ml RNase-free microcentrifuge tube (user supplied).
2. Gently brush a sterile, single-use cotton swab inside the nose or mouth.
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. Close tube and vortex gently. Incubate for 5 min at room temperature.
4. Using a pipette, transfer lysate into another 1.5ml RNase-free microcentrifuge tube (user supplied). Note the volume of the lysate. **Proceed to section 2.**

1.3 LYSATE PREPARATION FROM VIRUSES IN CULTURED CELLS

1.3.1 Cells Growing In a Monolayer

1. Aspirate media and wash cell monolayer with an appropriate amount of PBS. Aspirate PBS.
2. Add 350µl of Lysis Buffer RX directly to cell culture plate.
   Note: This volume of lysis buffer can be used for a culture plate of ~35mm in diameter or up to one well of a 6-well culture dish.
3. Lyse cells by gently tapping culture plate and swirling buffer around plate surface for 5 min.
4. Transfer lysate to a 1.5ml RNase-free microcentrifuge tube (user supplied). **Proceed to section 2.**

1.3.2 **Cells Growing In Suspension and Lifted Cells**

1. Transfer cell suspension to a 1.5ml RNase-free microcentrifuge tube (user supplied) and centrifuge at no more than 200 x g for 10 min to pellet cells.
2. Carefully decant the supernatant to ensure that the pellet is not dislodged. A few microliters of media may be left behind with the pellet in order to ensure that the pellet is not dislodged.
3. Add 350µl of Lysis Buffer RX to the pellet. Lyse cells by vortexing for 15s. Ensure the entire pellet is completely dissolved before proceeding to the next step. **Proceed to section 2.**

1.4 LYSATE PREPARATION FROM VIRUSES IN SUSPENSION

1.4.1 **Protocol**

1. Transfer up to 100µl of viral suspension to a 1.5ml RNase-free microcentrifuge tube (user supplied).
2. Add 350µl of Lysis Buffer RX. Lyse cells by vortexing for 15s. Ensure that mixture becomes transparent before proceeding with the protocol. **Proceed to section 2.**

1.5 LYSATE PREPARATION FROM VIRUSES IN MAMMALIAN TISSUE

1.5.1 **Protocol**

1. Determine amount of tissue by weighing.
2. Transfer the tissue into a mortar that contains enough liquid nitrogen to cover the sample. Grind thoroughly using a pestle.
3. Allow the liquid nitrogen to evaporate, without allowing the tissue to thaw.
4. Add 600µl of Buffer RX to the tissue sample and continue to grind until the sample has been homogenized. Homogenize by passing the lysate through a nuclease-free 20 gauge (0.9mm diameter) syringe needle (user supplied) 5-10 times.
5. Using a pipette, transfer the lysate into a 1.5ml RNase-free microcentrifuge tube (user supplied).
6. Spin the lysate for 2 min to pellet any cell debris. Transfer the supernatant to another 1.5ml RNase-free microcentrifuge tube. Note the volume of the supernatant/lysate. **Proceed to section 2.**

SECTION 2. GENOMIC DNA REMOVAL FROM ALL TYPES OF LYSATE

2.1 **Protocol**

1. Assemble an ISOLATE II **Genomic DNA Removal Column** (white ring) with a provided Collection Tube.
2. Apply up to 600µl of lysate prepared from section 1 onto the column and centrifuge at 1 min for 14,000 x g.
   Note: Ensure the entire lysate volume has passed into the Collection Tube by inspecting the column. If the entire lysate volume has not passed through, centrifuge for an additional 1 min at 14,000 x g.
3. Transfer the flow-through into a sterile RNase-free 1.5ml microcentrifuge tube (user supplied) for Total RNA Purification (see section 3). The flow-through contains the RNA and should be stored on ice or at -20°C until the Total RNA Purification protocol is carried out.
4. Discard the column with the bound genomic DNA. **Proceed to section 3.**

SECTION 3. TOTAL RNA PURIFICATION FROM ALL TYPES OF LYSATE

3.1 **Binding RNA to Column**

1. To every 100µl of collected flow-through from section 2.1, add 60µl of 96-100% ethanol. Mix by vortexing. For example, for 200µl of flow-through, add 120µl of ethanol.
2. Assemble an ISOLATE II **RNA Column** (black ring) with a provided Collection Tube.
3. Apply up to 600µl of the ethanolic lysate onto the column and centrifuge for 1 min at ≥3,500 x g.
   Note: Ensure the entire lysate volume has passed into the Collection Tube by inspecting the column. If the entire lysate volume has not passed through, centrifuge for an additional minute at 14,000 x g.
4. Discard the flow-through. Reassemble the spin column with its Collection Tube.
5. Depending on the lysate volume, repeat steps 3 and 4 as required.
6. **Optional:** The ISOLATE II FFPE RNA/DNA Kit purifies total RNA with minimal amounts of genomic DNA contamination. However, for sensitive applications, an optional on-column DNA removal protocol is provided in Appendix A of the full manual. DNase I treatment should be performed at this point in the protocol with the supplied DNase I.

3.2 RNA Column Wash

1. Apply 400µl of Wash Buffer W1 to the RNA Column and centrifuge for 1 min at 14,000 x g.
   Note: Ensure the entire wash buffer volume has passed into the Collection Tube by inspecting the column. If the entire wash volume has not passed through, centrifuge for an additional 1 min at 14,000 x g
2. Discard the flow-through and reassemble the spin column with the Collection Tube.
3. Repeat steps 1 and 2 to wash column a second time.
4. Wash column a third time by adding 400µl of Wash Buffer W1 and centrifuge for 1 min at 14,000 x g.
5. Discard flow-through and reassemble spin column with its Collection Tube.
6. Centrifuge for 2 min at 14,000 x g in order to dry the column thoroughly. Discard the Collection Tube.

3.3 RNA Elution

1. Place the RNA Column into a fresh 1.7ml Elution tube (supplied).
2. Add 50µl of RNA Elution Buffer to the column.
3. Centrifuge for 2 min at 200 x g, followed by 1 min at 14,000 x g. Note the volume eluted from the column. If the entire volume has not been eluted, spin column for an additional 1 min at 14,000 x g to elute the RNA.
   Note: For maximum RNA recovery, apply a second volume of 50µl RNA Elution Buffer and repeat step 3. Alternatively, re-apply the first eluate onto the column and re-elute into the same microcentrifuge tube (for higher concentration).

3.4 Storage of RNA

Store isolated RNA at -20°C for up to three days or at -80°C (recommended) for long term storage.

PRODUCT	PACK SIZE	CAT NO.
ISOLATE II Biofluids RNA Kit (Phenol free)	50 Preps	BIO-52086

Technical Support
The troubleshooting guide is provided in the online protocol.
For technical support please email us at tech@bioline.com
or visit www.bioline.com