PCRun® Sample Prep

RNA Extraction Kit

Cat. No.30PRE308

User manual - For the extraction of RNA for the use with PCRun® RNA Molecular Detection Kits.

INTENDED USE

The PCRun® RNA Sample Prep is intended for the extraction of RNA from fresh recently collected samples of non-coagulated whole blood (EDTA), buffy coat, cells, tissues, urine, throat and nose swabs in approximately 5 minutes. Protocols for extraction from various samples can be found in this manual.

PRINCIPLE

The PCRun® RNA Sample Prep is based on Zymo Research technology which involves lysis of cells, adhesion to a selective membrane, removal of inhibitors with wash solution and elution of the RNA.

STORAGE AND HANDLING

- Room temperature (21-25°C).
- Avoid exposure to direct sunlight.
- Do not use beyond expiration date stated on the package label.
- Do not freeze or expose to extreme temperatures.

EQUIPMEND AND REAGENT REQUIRED BUT NOT INCLUDED IN THIS KIT

- Accurate laboratory pipette with aerosol barrier tips (20 µl and 1000 µl range).
- Microcentrifuge suitable for Eppendorf tubes and which can reach a speed of 10,000 x g.
- Protective disposable laboratory gloves.
- Fine tipped indelible marker.
- For swab samples: sterile scissors or scalpel.
- Sterile physiological saline.

PRECAUTIONS

Proper handling and use of RNase free materials will eliminate degradation of RNA and introduction of RNases.

- Perform RNA extraction in an area separate from the area used for reaction preparation.
- Always employ dedicated pipettors and RNase free barrier tips when working with RNA.
- Clean disposable laboratory gloves must be worn while performing the extraction procedure.
- Do not use kit if any of the components are damaged.
- Each component in this kit is suitable for use only with a specific lot number. Do not mix components from different lot numbers.
- Use accepted laboratory procedures for working with RNA.
- All molecular waste should be disposed of in proper biohazard containers.

Extracted RNA can be stored for 24 hrs at 4°C and >24 hrs at -70°C.

SAMPLE COLLECTION, STORAGE AND TRANSPORT

For optimal results, recently acquired samples are recommended. If not processed shortly following collection samples can be maintained at 4°C for up to 24 hrs or -70°C for an extended period of time. For additional information on collection and maintenance of samples, please refer to Biogal's leaflet "Essential Instructions to Follow to Ensure Valid Results."

KIT CONTENTS

Contents	Amount
Bottle containing Viral RNA Buffer	1 bottle / 8 ml
Bottle containing Viral Wash Buffer	1 bottle / 4.5 ml
Vial containing RNA Elution Buffer	1 vial / 0.2 ml
Zymo Spin IC Columns	8
Collection Tubes for collection of waste materials	16
1.5 ml Eppendorf Tubes	8
2 ml Microcentrifuge Tube	8
Instruction manual	1

RNA EXTRACTION PROTOCOLS

A. General Protocol

The general protocol does not contain exact volumes in the instructions. It is designed to allow the user to modify the extraction method according to the potential sample volumes and types. For specifics instructions for blood, buffy coat, swabs and urine please refer to sections B-D below. Refer to the Rapid Instructions accompanying the kit for a pictorial explanation of each step.

1. Place the sample into the **2 ml Microcentrifuge Tube** supplied with the kit.
2. Add 3 volumes of **Viral RNA Buffer** to the sample and mix well by vortex or careful agitation.
3. Place a **Zymo-Spin™ IC Column** into a **Collection Tube**. Label the lid of the column clearly with the sample code.
4. Transfer the sample to the **Zymo-Spin™ IC Column** in the **Collection Tube** and centrifuge for 1 min. at a speed of 10,000 x g. Ensure the entire sample has passed through the filter into the **Collection Tube**. If remnants of the sample remain in the column, repeat the centrifugation.
5. Place the **Zymo-Spin IC Column** into a new **Collection Tube** (supplied with the kit). Discard the used **Collection Tube** and its contents into a suitable biohazard container.
6. Add 500 µl **Viral Wash Buffer** to the column and centrifuge for 2 min. at 10,000 x g. Take note that the entire volume of the buffer has passed through the column. If buffer is still visible in the column repeat the centrifugation step.
7. Carefully transfer the column, avoiding contact with the contents of the **Collection Tube**, to a 1.5 ml Eppendorf tube supplied with the kit. Discard the **Collection Tube** with its contents into a biohazard container. Label the lid of the Eppendorf clearly with the sample code.
8. Add 20 µl **RNA Elution Buffer** directly to the center of the column.
9. Centrifuge for approximately 30 seconds. Immediately close the tube and place on ice.
10. Eluted RNA should be used immediately or stored at -70°C.

B. Protocol for Buffy Coat or Whole Blood

The protocol is designed for the isolation of viral RNA from 200 µl of non-coagulated whole blood collected in EDTA or the buffy coat fraction isolated from a whole blood sample. If the volume of the sample is less than 200 µl, add physiological saline to the sample to reach the target volume.

1. Place the sample into a 2 ml Microcentrifuge Tube (200 µl - whole blood or isolated buffy coat with physiological saline).
2. Add 600 µl of Viral RNA Buffer to the sample and mix well.
3. Continue from step 3 of the **General Protocol** seen above.

C. Protocol for Conjunctival, Deep Pharyngeal and Deep Nasal Swabs

1. Add 600 µl **Viral RNA Buffer** and 200 µl physiological saline into a **2 ml Microcentrifuge Tube** supplied with this kit.
2. Using sterile scissors or disposable scalpel cut the swab tip into the **Microcentrifuge Tube** and mix vigorously. Sterilize the cutting instrument after use.
3. Place a **Zymo-Spin™ IC Column** into a **Collection Tube**. Label the lid of the column clearly with the sample code.
4. Carefully remove the **Viral RNA Buffer/Sample Mix** from the **Microcentrifuge Tube** and transfer it to the **Zymo-Spin™ IC Column** in the **Collection Tube.** Centrifuge for 1 min. at a speed of 10,000 x g. Ensure the entire sample has passed through the filter into the **Collection Tube**. If remnants of the sample remain in the column, repeat the centrifugation.
5. Continue from step 5 of the **General Protocol** above.

D. Protocol for Urine Samples

1. Add 900 µl Viral **RNA Buffer** and 300 µl urine sample into a **2 ml Microcentrifuge Tube** supplied with this kit and mix well by vortex or vigorous agitation.
2. Place a **Zymo-Spin™ IC Column** into a **Collection Tube** and label clearly with the sample code.
3. Transfer **half** of the buffer/urine mixture from the **Microcentrifuge Tube** to the **Zymo-Spin™ IC Column** in the **Collection Tube** and centrifuge for 1 min. at a speed of 10,000 x g. Ensure the entire sample has passed through the filter into the **Collection Tube**. If remnants of the sample remain in the column, repeat the centrifugation.
4. Discard the flow-through into a biohazard container and replace the column into the **Collection Tube**.
5. Repeat step 3 of this protocol with the remainder of the sample.
6. Continue from step 5 of the **General Protocol** above.

Appendix:

Transformation of g (RCF) to rpm

g=(1.118 x 10-5) RS2

g= Relative Centrifugal Force (RCF)

S=Speed of the centrifuge (rpm)

R=Radius of the rotor (cm)

For assistance please contact Biogal Galed Labs Acs.

Manufacturer: Zymo Research

Biogal Galed Labs. Acs. Ltd.
TEL: +972 (0)4 9898605 FAX: +972 (0)4 9898690
email: info@biogal.co.il www.biogal.co.il
Kibbutz Galed, 1924000 - Israel