PCRun® Sample Prep

DNA Extraction Kit

Cat. No.30PRE208

User Manual – For extraction of DNA for downstream use with PCRun Molecular Detection Kit

INTENDED USE

PCRun Sample Prep is intended for the extraction of DNA from fresh and frozen whole blood, bone marrow, body fluids, concentrated urine, swabs and fine needle aspirations (FNA), in approximately 15 minutes. The kit can be employed with samples collected in EDTA, heparin and citrate.

PRINCIPLE

PCRun Sample Prep is based on Zymo technology which involves lysis of cells, adhesion to a selective membrane, removal of inhibitors by 2 fast washes and elution of DNA.

STORAGE AND HANDLING

- Room temperature (21-25^{0}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

RECOMENDATIONS

- If the DNA prewash buffer (**B**) is not clear, warm the buffer to 30-37^{0}C for 30 minutes and mix by inverting. Do not microwave!

Precautions:

- Clean 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 sanitary procedures designated for biological and molecular waste when handling and disposing of kit components.
- Perform DNA extraction in an area separate from the area used for reaction preparation.

EQUIPMENT TO BE SUPPLIED BY USER

- Microcentrifuge which reaches a speed of 10,000 x g
- Timer
- Protective laboratory gloves
- Fine tipped indelible marker

SAMPLE COLLECTION, STORAGE AND TRANSPORT

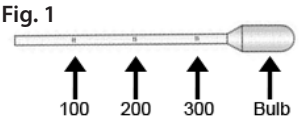
For optimal results recently acquired samples are recommended. Samples can be maintained at 4^{0}C for up to 24 hours or -20^{0}C for an extended period of time. The mode for processing different types of samples can be found in the specific relevant protocols.

KIT CONTENTS

Catolog Number	Contents	Amount
03204282	Disposable pastettes for transfer of liquids	35
30304150	Vials containing Genomic Lysis Buffer (**A**) (contains mercaptoethanol)	8 vials / 400 µl
30304515	Bottle containing DNA Pre-Wash Buffer (**B**)	1 bottle / 2 ml
30304250	Bottle containing gDNA Wash Buffer (**C**)	1 bottle / 4.5 ml
30304410	Drop bottle containing DNA Elution Buffer (**D**)	1 bottle / 1 ml
03205250	Zymo Spin Columns	8
03205100	Collection Tubes for collection of waste materials	16
04400214	1.5 ml Tubes for collection of extracted DNA	8
33PRE208	Instruction manual	1
33QRP108	Quick Reference Protocol Card	1

General information

Fig. 1

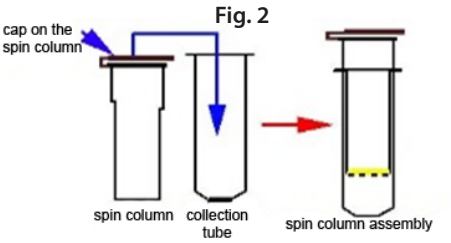


DNA Extraction Protocols

Disposable graduated plastic pastettes are supplied with the kit for transfer of the various liquid components. The tip is marked with three lines representing volumes of 100 µl (Fig.1).
In order to transfer a volume of sample or buffer, the bulb should be depressed to expel air, the tip of the pastette dipped into the liquid and the pressure on the bulb released until the fluid reaches the desired line. The liquid is released into the target receptacle by applying pressure to the bulb. The pastettes are disposable and should be used only as indicated in the instructions.

A. Extraction of DNA from whole blood, bone marrow, serum and plasma

Fig. 2



1. Using a clean pastette, measure 100 µl sample (1 line) and add to a vial containing Buffer **A**. Mix well. Allow to stand at room temperature for 5 min. Discard the used pastette in a suitable biohazard container.
2. Using a clean pastette, transfer the sample/buffer **A** mixture to a Spin Column which has been placed into a collection tube (Fig. 2).
3. Centrifuge at 10,000 x g for one minute. Dispose of the collection tube and place the column into a clean collection tube.
4. Using a clean pastette add 200 µl (2 lines) of Buffer **B** to the spin column. Centrifuge at 10,000 x g for one minute. There is no need to empty the collection tube.
5. Using a clean pastette add 500 µl Buffer **C** to the spin column (fill the pastette to the third line, add the buffer to the spin column and repeat the action by filling the pastette to the second line). Centrifuge at 10,000 x g for 3 minutes.
6. Transfer the spin column to a clean 1.5 ml tube. Add 1 full drop (50 µl) Buffer **D** to the spin column. Incubate 2 minutes at room temperature. Centrifuge at 10,000 x g for 30 seconds. The DNA will be released into the 1.5 ml tube. Discard the **spin column** and close the lid of the 1.5 ml tube. Label the tube clearly with a sample code. The extracted DNA should be used immediately for the PCRun reaction. If this is not practical, store the extract at 4°C for a time period of no longer than 24hrs.
7. It is recommended that freshly extracted DNA be used for PCRun reactions. DNA extracts can be maintained at -20°C for extended periods of time. Avoid multiple freezing and thawing.

B. Extraction of DNA from urine samples

DNA can be extracted from cells and pathogens present in urine samples. 2 ml of urine should be treated in the following manner for optimal DNA isolation.

1. Add 100 µl of 10X PBS (not provided) to each 1 ml of fresh urine and mix well.
2. Centrifuge at 10,000 x g for 10 minutes.
3. Discard the supernatant leaving the pellet at the botton of the tube.
4. Resupend the pellet in 100 µl of 1X PBS (not provided).
5. Extract DNA according to protocol **A** (see above).

C. Extraction of DNA from swabs samples.

DNA can be extracted from clinical samples which have been collected on swabs. It is important to thoroughly rub the infected area in order to collect sufficient tissue on the swab. Swabs which do not contain enough biological matter are not a good source for DNA purification.

1. Cut the area of the swab containing the biological material into small pieces and transfer to a vial of Buffer **A.**
2. Allow to stand at room temperature for 10 minutes mixing thoroughly several times during this time period.
3. Transfer the mixture to a Spin Column and continue as in protocol **A3** (above).

D. Extraction of DNA from Fine Needle Aspirations

This protocol can be used for fine needle aspirations (FNA) such as lymph node or spleen. The tissue must be homogenize prior to extraction. Cut tissue into small pieces and squash against the side of the collection tube until it loses its entire integrity.
1. Mechanical homogenization can be performed on fresh or frozen tissue in **400 µl of Genomic Lysis Buffer** and mix well. Allow to incubate at room temperature for 5 min.
2. Centrifuge the lysate at top speed (10,000 x g) for 5 minutes. Make sure not to disturb the pelleted debris.
3. Using a clean pastette to transfer the supernatant to a Spin Column in a Collection Tube and continue as in Protocol **A3** (above).

Appendix:
Transformation of g (RCF) to rpm
$g=(1.118 \times 10^{-5}) RS^2$
g= Relative Centrifugal Force (RCF)
S=Speed of the centrifuge (rpm)
R=Radius of the rotor (cm)

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