PCRun® Sample Prep

DNA Extraction Kit

Cat. No.30PRE216

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

- Accurate laboratory pipette with aerosol barrier tips (100, 200 ans 500 µl)
- 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

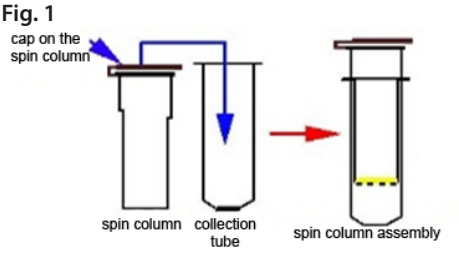
Catolog Number	Contents	Amount
30304150	Genomic Lysis Buffer (**A**) (contains mercaptoethanol)	16 vials/400 µl
30304515	DNA Pre-Wash Buffer (**B**)	2 bottle / 2 ml
30304250	gDNA Wash Buffer (**C**)	2 bottle / 4.5 ml
30304410	DNA Elution Buffer (**D**)	2 drop bottles/1 ml
03205250	Zymo Spin Columns	16
03205100	Collection Tubes for collection of waste materials	32
04400214	1.5 ml Tubes for collection of extracted DNA	16
33PRE216	Instruction manual	1
33QRP116	Quick Reference Protocol Card	1

DNA EXTRACTION PROTOCOLS

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

1. Using a an accurate laboratory pipette and sterile barrier tips, add 100 µl blood to a vial containing the Genomic Lysis Buffer (**A**) (4:1). Allow to stand at room temperature for 5 min. Discard the used tip in a suitable biohazard container.
2. Transfer the sample/buffer **A** mixture to a Spin Column which has been placed into a collection tube (Fig. 1).
3. Centrifuge at 10,000 x g for 1 minute. Dispose of the collection tube and place the column into a clean collection tube.
4. Add 200 µl 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. Add 500 µl Buffer **C** to the spin column. 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 of 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.

Fig. 1



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 each1 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 containing 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 ul of Genomic Lysis Buffer. Allow to incubate at room temperature for 5 min and mix well.
2. Centrifuge the lysate at top speed (10,000 x g) for 5 minutes. Make sure not to disturb the pelleted debris.
3. Transfer the supernatant to a Spin Column in a Collection Tube and continue as 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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