PCRun® Sample Prep

Rapid DNA Extraction Kit

Cat. No.30PRE108

User Manual – For *in vitro* extraction of DNA for downstream use with PCRun® Molecular Detection Kit

INTENDED USE

PCRun® Sample Prep Rapid DNA Extraction Kit is intended for the extraction of DNA from blood, bone marrow and concentrated urine. Please read the PCRun® reaction instruction manual to determine which sample type is indicated for the chosen diagnostic kit. The kit contains all the disposable components required for performing extraction of DNA that is compatible for use with PCRun® molecular kits. DNA extraction can be accomplished with the use of a microcentrifuge or manual displacement with a 10 ml syringe supplied. Both methods are described in this manual.

STORAGE AND HANDLING

- Storage 2-25^{0}C (room temperature or refrigerated).
- Avoid exposure to direct sunlight.
- Do not use beyond expiration date stated on the package label.
- Do not freeze or expose to extreme temperatures.

Precautions:

- Clean laboratory gloves must be worn while performing the test.
- Open reaction receptacle only prior to use.
- Do not use kit if any of its components are damaged.
- Each component in this kit is suitable for use only with a specific lot number. Components have been quality control approved as a standard batch unit. 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 PCRun® reaction preparation.

PRINCIPLE

The PCRun® Sample Prep Rapid DNA Extraction Kit is based on heat denaturation of proteins and column separation of DNA. Isothermal amplification inhibitors are removed during the process and the DNA extract is released into a buffer which is compatible for downstream reactions.

LIMITATIONS

PCRun® sample prep is designed for use with Biogal PCRun® molecular detection kits.

KIT CONTENTS

Catalog Number	Contents	Amount
31000130	PCRun® columns containing PCRun® Extraction Buffer - Red Cap	8 columns/50 µl
31031001	Collection vials containing PCRun® Dilution Buffer - Green Cap	8 vials/ 380µl
03200050	Disposable plastic capillary tubes - 50 µl	1 package - 10 tubes
03200030	Luer lock caps	8
03200040	10 ml syringe	1
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EQUIPMENT TO BE SUPPLIED BY USER

- Microcentrifuge which can maintain 1500 X g (Centrifuge protocol only)
- Timer
- Heat block which maintains 95^{0}C – compatible with 1.5 ml Eppendorf tubes (Can be supplied by Biogal).
- Protective laboratory gloves
- Fine tipped permanent marker

SAMPLE COLLECTION, STORAGE AND TRANSPORT

The kit is suitable for the extraction of nucleic acid from 50 µl of whole blood, bone marrow or 1 ml of urine. Check with the PCRun® instruction manual to determine what sample type is suitable for diagnostic purposes. **Please note:** 10X PBS and 1X PBS are supplied with PCRun® kits in which urine samples are recommended for analysis.

Blood

Whole blood can be collected in EDTA, heparin or sodium citrate.

Urine Sediment

One hundred microliters of 10X PBS should be added to 1 ml of fresh urine. The sample should then be cetrifuged at high speed (10,000 x g) for ten minutes. The supernatant is discarded and the solid material (pellet) found at the bottom of the test tube should be resuspended in 50 ul of 1X PBS prior to DNA extraction. The concetrated urine samples can be stored frozen.

PROTOCOL - PCRun® DNA EXTRACTION

Turn on the heat block and adjust the temperature to 95°C. When the target temperature has been reached begin the DNA extraction.

Prior to beginnig the DNA extraction, tap the column lightly on the table counter to ensure that all of the extraction buffer is located above the column filter.
Do the same with the collection vials containing the dilution buffer.

Using the 50 µl capillary tube add 50 µl of well mixed sample to the PCRun® extraction buffer found in the column (red cap). (Fig 1)

1. Close the column and mix by tapping lightly on the side of the column.
2. Place the column into the appropriate hole of the preheated block **(Fig 2).**
3. Incubate in the heat block (95°C) for 5 min.
4. Remove the column from the heat block. Loosen the red cap. Cool the column with the sample for 1 min at room temperature **(Fig 3).**
5. When using the heat block for a PCRun® reaction, cool the instrument to 60°C by lowering the target temperature on the heat block control screen. The cooling process can take up to 15 minutes. To expedite the process the block can be removed with the aid of the Block Lifter supplied with the instrument and placed in a cold environment for several minutes. Avoid contact with skin or clothing when removing the hot block. Continue to centrifuge method (6a) or syringe method (6b).

6a. Centrifuge method:

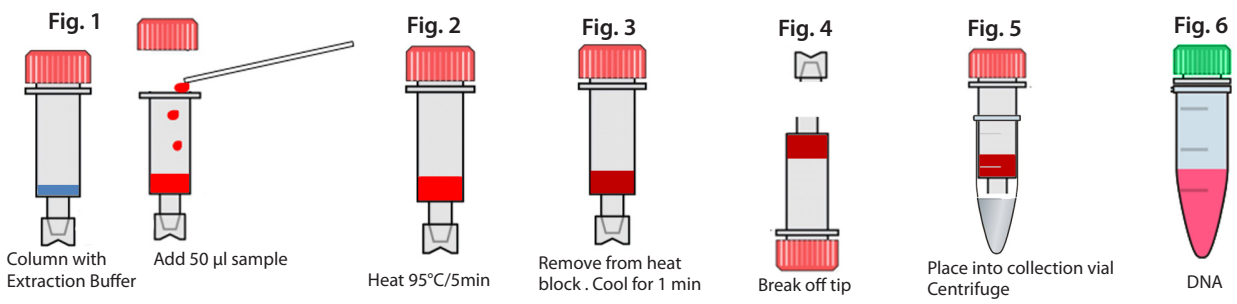
- Tighten the red cap. Invert the column and break off the column base (**Fig. 4**). Avoid cross contamination between columns when analyzing more than one sample!
- Place the column into a 1.5 ml collection vial containing PCRun® Dilution Buffer (green cap) **(Fig 5)**. Save the green cap for later use.
- Centrifuge the column/collection vial at 1,500 x g for 1 min. Continue with step 7.

6b. Syringe method:

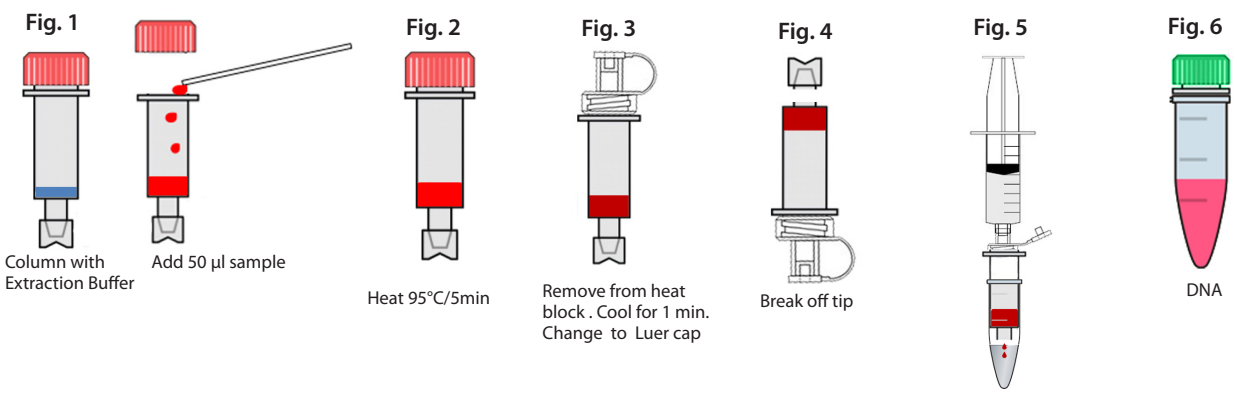
- Remove the red cap and replace it with the luer lock cap (**Fig 3**). Firmly screw the luer lock cap to the column and close the cap opening with the attached plug.
- Invert the column and break off the column base (**Fig. 4**). Avoid cross contamination between columns when analyzing more than one sample!
- Place the column into a 1.5 ml collection vial containing PCRun® Dilution Buffer (green cap). Save the cap for later use.
- Unplug the luer lock cap.
- Hold the syringe firmly and displace 10 ml of air by pulling the plunger to the 10 ml line (open position).
- While the plunger is in the open position firmly attach the 10 ml syringe to the spout. Make sure that all attachments are air tight **(Fig 5)**.
- Apply gentle pressure on the syringe plunger. Continue to apply pressure until resistance is felt. Observe the area between the tip of the column and the dilution buffer. During this stage a small volume of liquid, in the form of a drop or bubbles, will fall into the Dilution Buffer. If liquid is not released from the column; detach the syringe and repeat the last two steps **(Fig 5)**.

7. Detach the collection vial from the column and discard the column in a biological waste bin.
8. Cap the tube with the green cap and thoroughly mix the contents of the collection vial now containing the extracted **DNA (Fig 6)**.
9. The extracted **DNA** is suitable for use in most PCRun® reactions. 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.

6a. Centrifuge Method



6b. Syringe Method



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