CURRENT

Category: General SOP for Extraction of genomic DNA from whole blood in Genetics laboratory.

Title: Extraction of genomic DNA from whole blood by kit method in the Department of Clinical Pharmacology, K.E.M Hospital, Mumbai.

SOP No.: Pre PCR-Method- 06

Date first effective: 1st January 2025 **Review date:** 31st December 2025

Department of Clinical Pharmacology, 1st floor, New MS building Seth GS Medical College & KEM Hospital, Parel, Mumbai 400012.

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Version: 06

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Purpose: This standard operating procedure (SOP) describes the method for extraction of genomic DNA from whole blood by kit method

Scope: This SOP is limited for extraction of genomic DNA from whole blood by kit method

Responsibilities:

Primary Responsibility: Divya Bhere
Lab Technician
30/Dec/2024

Secondary Responsibility: Dr.Sheetal Kudtarkar
Project Scientist
30/12/24

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Detailed Instructions:

Important: This procedure should be carried out wearing sterile gloves

Reagents preparation:

- Proteinase K: Add 1.34 mL double distilled water to lyophilized Proteinase K powder.
- Wash buffer 1: Add 8mL absolute ethanol to concentrated wash buffer1 to make working wash buffer.
- Wash buffer: Add 40mL absolute ethanol to concentrated wash buffer to make working wash buffer.

Procedure:

1. Pipette 20µL proteinase K into the bottom of a 1.5 mL micro -centrifuge tube, to it add 200µL blood/ body fluid sample or buffy coat from 1ml blood (equilibrated to room temperature).
2. Add 200µL buffer FABG to the samples and vortex the mixture vigorously (30 sec). Incubate samples at 70^0C for 10-15 mins.
3. Add 200µL ethanol (96-100%) to each sample and vortex again.
4. Pipette the mixture from step 3 into the DNA Sure Blood Mini Column placed in a collection tube. Centrifuge 1 min at 11000 × g (see below). Discard collection tube with flow through.
5. Place the FABG Mini Column into fresh collection tube (2mL) and add 400µL W1 Buffer. Centrifuge 1 min at 11000 × g. Discard the collection tube with flow through.
6. Place the FABG Mini Column into fresh collection tube (2mL) and add 700 µL WASH buffer. Centrifuge 1 min at 11000 × g. Discard flow through and reuse collection tube.
7. Place the FABG Mini Column back into the collection tube and centrifuge for 1 min at 11000 × g
8. Place the FABG Mini Column in a 1.5mL micro-centrifuge tube and add 100µL preheated elution buffer (70^0C). Dispense buffer directly onto silica membrane. Incubate at room temperature for 1min. Centrifuge for 1min at 11000 × g.
9. Flow through contains the DNA. Store at 4^0C.

Note: The Relative Centrifugal Force (RCF) is expressed in units of gravity (time's gravity or × g). Many microcentrifuges only have settings for speed (revolutions per minute, RPM), not relative centrifugal force. Consequently, a formula for conversion is required to ensure that the appropriate setting is used in an experiment. The relationship between RPM and RCF is as follows:

$$g = (1.118 \times 10^{-5}) r S^2$$

Where 'g' is the relative centrifugal force, 'r' is the radius of the rotor in centimeters, and 'S' is the speed of the centrifuge in revolutions per minute.

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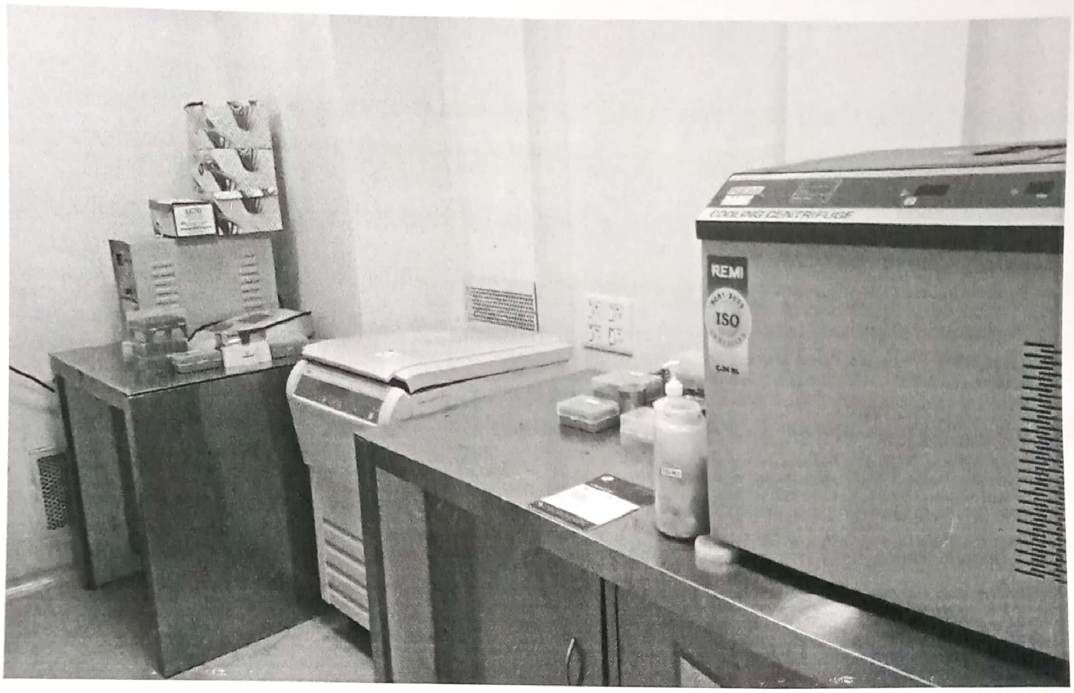
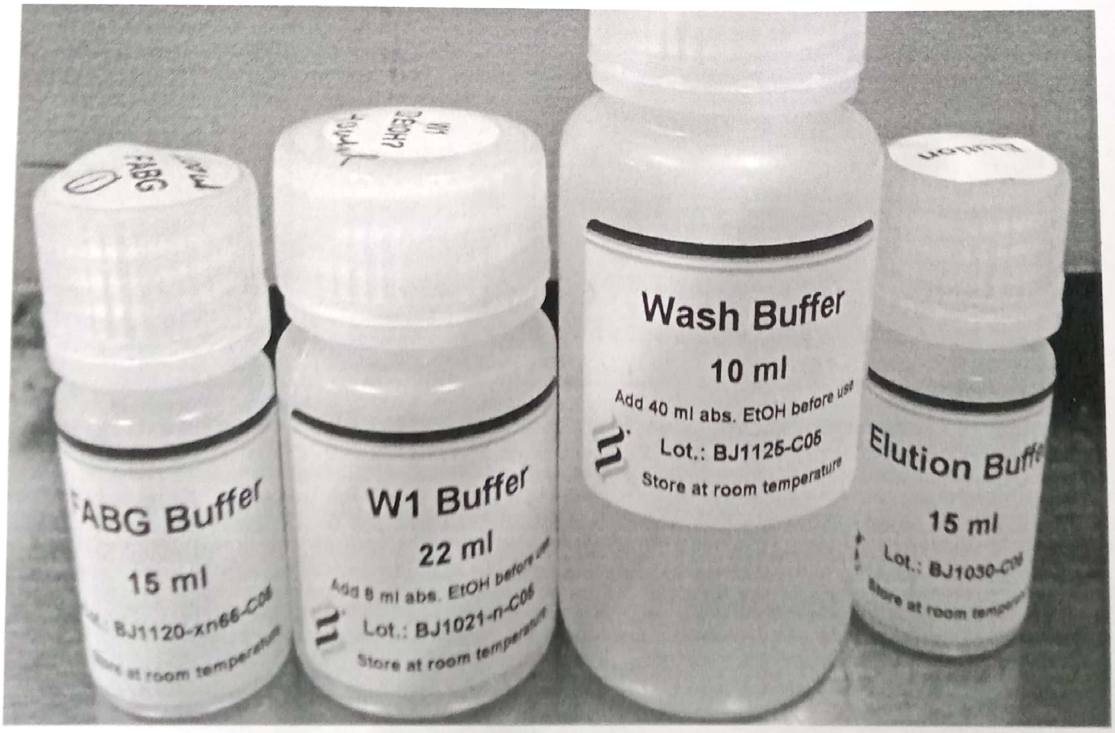


DNA extraction room



Reagents used

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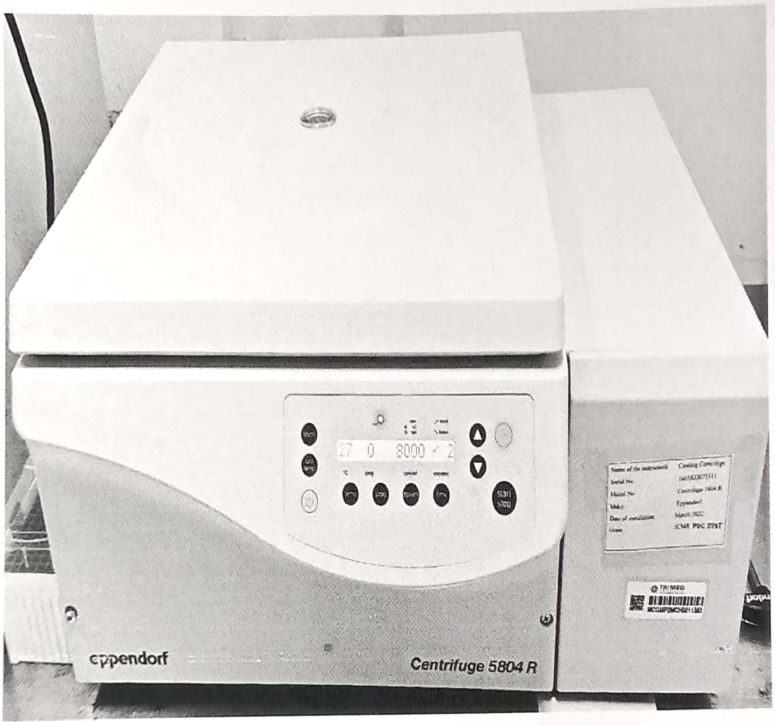
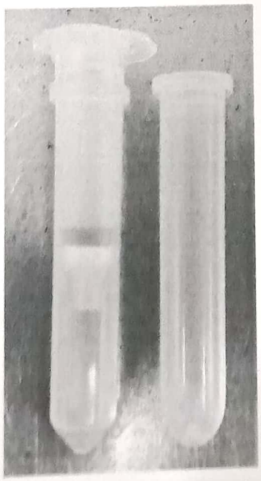


Cooling centrifuge



Column & Collection tube used for DNA extraction