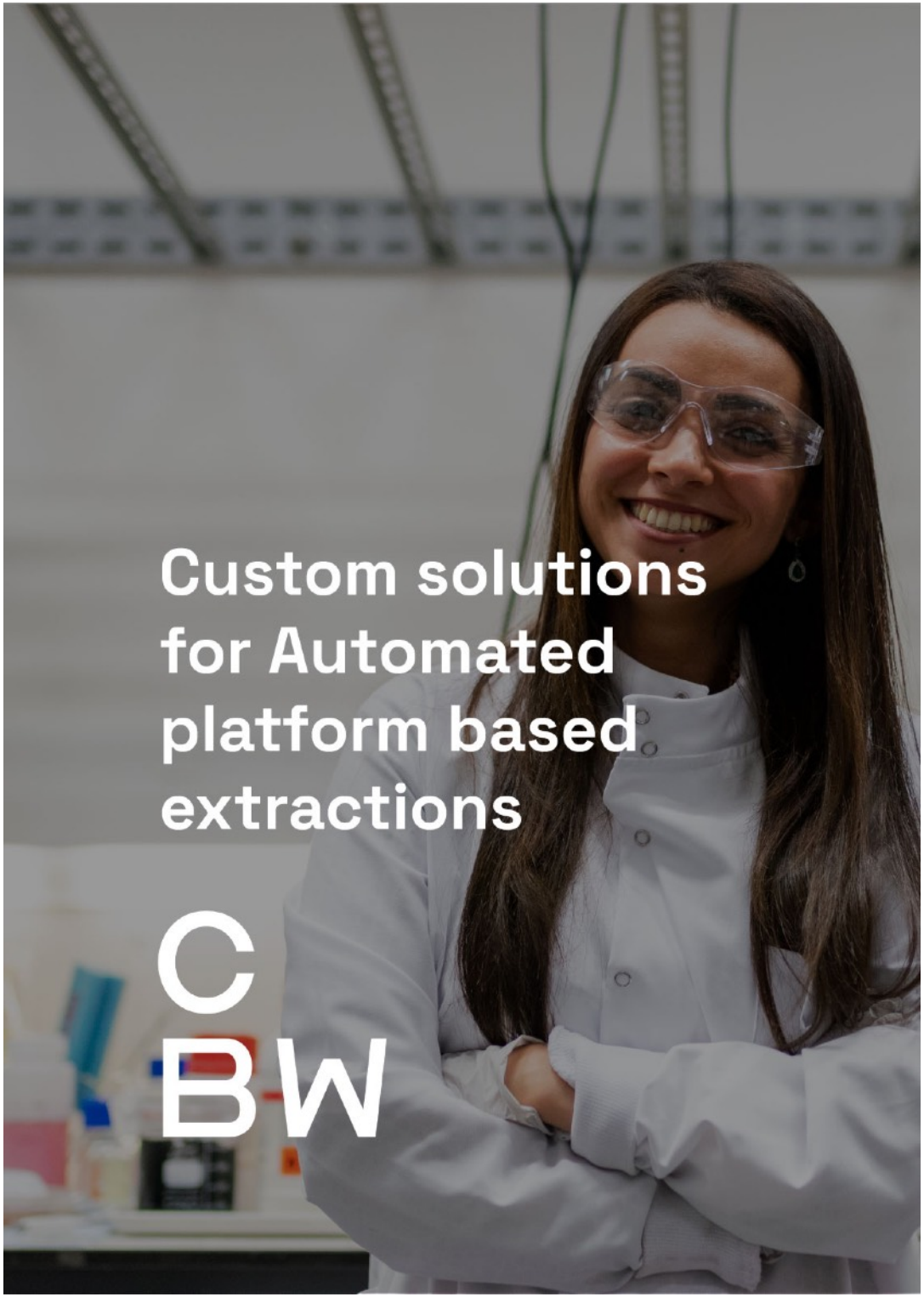

Standard Operation Procedure

BLOOD GENOMIC DNA EXTRACTION USING CAMSPIN COLUMN CBWC025

Introduction

Nucleic acids, DNA and RNA, hold valuable biological information. Nucleic Acid Amplification Tests (NAAT) are rapid, specific and more sensitive compared to culture-based tests and more specific than serology-based testing. Nucleic Acid Testing (NATs) are employed in detection of various diseased conditions like cancer, SNP based genetic disorders, and various chromosomal aberrations based genetic disorders. Genomic DNA is also employed in genotyping and genome sequencing using NGS platforms-based applications

A successful nucleic acid extraction forms the crux of the NAT assay. Our nucleic acid extraction kits utilize chaotropic salts for effective denaturation and deproteinization of interfering nuclear-protein complexes, inactivation of endogenous nuclease activity and enhancing binding of nucleic acid to the silica molecules for effective extraction of DNA to be used for various downstream applications.

Storage condition

DNA extraction kit can be kept at room temperature.

Kit contents

Contents	Quantity	Storage
Lysis Buffer	8 ml	Room temperature
Binding Buffer	3 ml	Room temperature
Wash Buffer I	5 mL	Room temperature
Wash Buffer II	6 ml	Room temperature
Nuclease Free Water	2 ml	Room temperature
Proteinase K	10 mg	Store at -20°C after reconstitution
Proteinase K Diluent	0.7 mL	Room temperature
CamSpin Columns	10 numbers	Room temperature

How to start

Items required by the user but not provided in the kit:

Equipment

1. Table-top micro-centrifuge (with rotor for 1.5 mL and 2 mL tubes).
2. Vortex mixer
3. Dry bath
4. Pipettes and pipette tips

Reagents to be supplied by the user

1. 96%- 100% Ethanol

Preparation of working solutions

1. Proteinase K: Add 500µL of Proteinase K diluent to 10 mg of Proteinase K provided and mix thoroughly. Store it at -20°C after reconstitution.

2. Wash buffer II**:** The Wash Buffer II is provided as a buffer concentrate. Add 4.5 mL of 96-100% ethanol* to 1.5mL of Wash Buffer concentrate.

*Do not use denatured alcohol which contains other undesirable substances like methyl-ethyl ketone or methanol.

Protocols

DNA extraction from blood

Note: The blood used for DNA extraction should be properly drawn and stored in an EDTA vacutainer and should have no clots.

1. Take 200µL of blood in a 1.5 mL microcentrifuge tube. Add 800µL of DNA lysis buffer and 10µL of Proteinase K. Mix thoroughly by inverting.
2. Vortex the tubes for about 1 minute. Incubate at 56°C for 10 mins.
3. Add 600µL of 100% ethanol to the tube and invert mix 6-7 times for effective mixing and precipitation. Incubate at room temperature for 10 minutes.
4. Add 650 µL of the lysate to the spin column. Centrifuge the columns at 13,000 rpm for 1 minute. Discard the flow through.
5. Load the remaining lysate into the spin column and spin down the columns at 13,000 rpm for 1 minute. Discard the flow through.
6. Add 300µL of the binding buffer to the column and incubate the column at room temperature for 10 minutes.
7. Spin down the columns at 13,000 rpm for 1 minute at room temperature. Discard the flow through.
8. Add 500µL of Wash buffer I to the spin column. Centrifuge the spin column at 13,000 rpm for 1 minute. Discard the flow through.
9. Add 500µL of Wash buffer II to the spin column. Centrifuge the spin column at 13,000 rpm for 1 minute. Discard the flow through.
10. Spin down the empty column again at 13,000 rpm for 1 minute to remove traces of ethanol. Discard the collection tube.
11. Place the spin column in a fresh RNAse-free microcentrifuge tube. Add 40µL of nuclease free water in the column and incubate the tube at room temperature for 10 minutes.
    (**Optional**: Use prewarmed nuclease-free water for higher yield of genomic DNA. Warm nuclease -free water at 56°C for 1-2 mins in a dry bath and use it for elution.)
12. Centrifuge the columns at 13,000 rpm for 1 minute at RT. Store the eluted DNA at -20°C.