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BIO BASIC INC.

96-Well Plate Blood Genomic DNA Mini-Preps Kit

BT92031 and BT92032

Version 5.0
ISO9001 Certified

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96-Well Plate Blood Genomic DNA Mini-Preps Kit

Product information for BT92031/BT92032:

Kit Contents

Components	BT92031 2 Plates	BT92032 5 Plates
PBS Solution	40 ml	100 ml
Universal Buffer CL	48 ml	120 ml
CW1 Solution (concentrate)	52 ml	2x65 ml
CW2 Solution (concentrate)	36 ml	2x45 ml
CE Buffer	40 ml	120 ml
Proteinase K	4.8 ml	12 ml
EZ-10 96 Well Plate	2	5
Deep Well Collection Plate	4	10
96 Storage Plate	2	5
Sealing film	8	20
Protocol	1	1

Note 1: Universal Buffer CL contains chaotropic salt. Avoid contact with skin and eyes.
Note 2: CW1 Solution and CW2 Solution are supplied as concentrates. Add **68ml** of **ethanol (96-100%)** to **52ml** of **CW1 Solution**; add **85ml** of **ethanol (96-100%)** to **65 ml** of **CW1 Solution**, add **84ml** of **ethanol (96-100%)** to **36ml** of **CW2 Solution**; add **105ml** of **ethanol (96-100%)** to **45ml** of **CW2 Solution** before use to obtain working solutions.

Storage and Stability

96 Well Plates and all buffers should be stored dry, at room temperature (15-25°C) and are stable for 1 year under proper storage. Proteinase K is supplied as 10 mg/ml solution, the solution can be keep at 4°C for 6 months, for long-term storage keep at -20°C.

Introduction

96 Well Plate blood genomic DNA minipreps kit is designed for rapid and high-throughout purification of genomic DNA from fresh or frozen anticoagulated blood. Samples are first lysed using proteinase K in an optimized buffer. The lysate is then loaded onto the 96 well EZ plate, DNA is selectively bound to the EZ membrane embed in the plate in the presence of high concentrations of chaotropic salt. During wash steps, protein and other impurities are removed and DNA is then eluted in water or low-salt buffer. Purified DNA typically has an A_{260}/A_{280} ratios of 1.7-1.9, and is highly suited for most downstream applications such as PCR, Southern blotting, RAPD and RFLP.

The purification procedure requires no phenol or chloroform extraction or alcohol precipitation, and involves minimal handling. The whole procedure takes only 20 minutes after sample preparation.

Note: The kit cannot distinguish different forms of DNA and will not be able to separate mitochondrial DNA from genomic DNA.

Features

- ü High quality of DNA, OD_{260}/OD_{280} of purified DNA is generally 1.8~1.9.
- ü Fast and effective. Fast and easy processing using a rapid spin-column format.
- ü Compatible with many downstream applications such as PCR, restriction digestion and hybridization.
- ü No phenol/chloroform extraction or ethanol precipitation is required.

Materials Supplied by User:

Microcentrifuge capable of at least 8,000 × *g*
Pipets and pipet tips
Vortexer
Ethanol (96-100%)
RNase A (20 mg/ml, Optional for RNA-free DNA)
Water bath for heating at 56°C

Before Starting:

This protocol is designed for purification of total DNA from fresh or frozen anticoagulated blood. All centrifugation steps are carried out at room temperature (15-25°C) in a microcentrifuge. It is strongly advised that you read this booklet thoroughly before starting. EZ-10 Column Blood Genomic DNA Purification Kit is designed to be simple, fast and reliable provided that all steps are followed diligently. Prepare all components, and have the necessary materials as outlined before starting.

Proteinase K is supplied in a ready-to-use solution form, but RNase A is not provided in this kit, if RNA-free DNA are required, please prepare RNA solution and see protocol to add the RNA removal step.

Check the Universal Buffer CL for salt precipitation before each use. If necessary, redissolve the precipitate by warming the solution at 56°C, then cool back down to room temperature before use.

CE Buffer is 10 mM Tris-HCl, 0.5 mM EDTA, pH 9.0. Water can be used as eluate in the final step if EDTA

should be avoided for the following applications, but it is not recommended if the pH of water is less than 7.0.
Preheat the water bath or rocking platform to 56°C.

Procedures

1. Sample Preparation.

A. No nucleated: Pipet 20 µl proteinase K into each well on a 96-Well Collection Plate. Add 50-100 µl anticoagulated blood. Adjust the volume to 220 µl with PBS. Continue with step 2.

B. Nucleated: Pipet 20 µl proteinase K into each well of 96-Well Collection Plate. Add 5–10 µl anticoagulated blood. Adjust the volume to 220 µl with PBS. Continue with step 2.

2. Add 200 µl Universal Buffer CL to the sample and covered with sealing film, mix thoroughly by vortexing. Incubate at 56°C for 10 min.

Note: If RNA-free genomic DNA is required, add 20 µl RNase A (20 mg/ml), mix by vortexing, and incubate for 2 min at room temperature before continuing with step 3.

3. Add 200 µl ethanol (96-100%) and covered with a new Sealing film, mix thoroughly by vortexing.

Note: Spin the 96-Well Collection Plate at room temperature before discard the Sealing film.

4. Transfer the mixture from step 3 (including any precipitate) into the EZ-10 96-Well Plate placed in a new 96-Well Collection Plate. Centrifuge at 5,000 *x g* (6,000 *rpm*) for 1 min. Discard the flow-through.

5. Add 500 µl CW1 Solution, and centrifuge for 1 min at 5,000 *x g* (6,000 *rpm*). Discard the flow-through.

Note: Check the label to ensure CW1 Solution was diluted with ethanol.

6. Add 500 µl CW2 Solution, and centrifuge for 1 min at 5,000 *x g* (6,000 *rpm*). Discard the flow-through.

Note: Check the label to ensure CW2 Solution was diluted with ethanol.

7. Place the empty EZ-10 96-Well Plate in the 96-Well Collection Plate and centrifuge for an additional 2 min at 5,000 *x g* (6,000 *rpm*) to dry the EZ-10 membrane. Discard flow-through and transfer the EZ-10 96-Well Plate to a 96-Well Storage Plate.

Note: It is important to dry the membrane of the EZ-10 96-Well Plate, since residual ethanol may interfere with subsequent reactions. This centrifugation step ensures that no residual ethanol will be carried over during the following elution.

8. Add 50-100 µl Buffer CE directly onto the center part of EZ-10 96-Well Plate membrane. Incubate at room temperature for 1 min, and then centrifuge for 1 min at 5,000 *x g* (6,000 *rpm*) to elute the DNA.

Note 1: Warm the Buffer CE to 60°C will increase the elution efficiency.

Note 2: Elution with more than 100 µl (e.g. 200 µl) increases the DNA yield, but the concentration will be lower.

Note 3: For maximum DNA yield, repeat elution once as described in this step.

Note 4: A new microcentrifuge tube can be used for the second elution step to prevent dilution of the first eluate.

Note 5: For maximum DNA concentration, use the eluate in the microcentrifuge tube for the second elution step.

Other Kits Available

EZ-10 Spin Column Plasmid DNA MiniPreps Kit

BS413 (50 Preps)
BS414 (100 Preps)
BS614 (250 Preps)

EZ-10 Spin Column PCR Products Purification Kit

BS363 (50 Preps)
BS364 (100 Preps)
BS664 (250 Preps)

EZ-10 Spin Column DNA Gel Extraction Kit

BS353 (50 Preps)
BS354 (100 Preps)
BS654 (250 Preps

PRODUCTS ARE INTENDED FOR BASIC SCIENTIFIC RESEARCH ONLY!
NOT INTENDED FOR HUMAN OR ANIMAL USE!

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