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BIO BASIC INC.

EZ-500 Spin Column Animal Genomic DNA Maxi-Preps Kit

PT92034

Version 4.0
ISO9001 Certified

20 Konrad Cres, Markham Ontario L3R 8T4 Canada
Tel: (905) 474 4493, (800) 313 7224 Fax: (905) 474 5794
Email: order@biobasic.com Web: www.biobasic.com

EZ-500 Spin Column Animal Genomic DNA Maxi-Preps Kit

Product information for PT92034:

Kit Contents

Components	PT92034, 4 Preps
Universal Buffer ACL	50 ml
Universal Buffer CL	60 ml
CW1 Solution	13 ml
CW2 Solution	9 ml
CE Buffer	20 ml
Proteinase K	6 ml
EZ-500 Column	4
50-ml Collection Tube	4
Protocol	1

Storage

EZ columns and all buffers should be stored dry, at room temperature (15-25°C) and are stable for 1 year under these conditions. Proteinase K is supplied as 10 mg/ml solution, the solution can be stored at 4°C for 6 months or at -20°C for long term.

Introduction

The kit is designed for a large scale isolation of high molecular weight and high quality genomic DNA from a wide variety of fresh or frozen animal tissue using a rapid maxi-preps spin column format. DNA in animal lysates is selectively absorbed on EZ-500 Maxi Spin Column, and other impurities don't bind on the column. No phenol extraction, no ethanol precipitations are required. The procedure is simple and rapid, it takes approx 20 minutes only. The kit does not contain toxic reagents. Purified Genomic DNA can be used for PCR, Real Time PCR and other downstream applications.

Features

- **ü** Fast. Uses a rapid spin-column format, the entire procedure takes 20 minutes only.
- **ü** High Quality of DNA. OD_{260}/OD_{280} ratio of purified DNA is generally > 1.8. Purified DNA can be used for PCR, Real Time PCR and other downstream applications.
- **ü** Suitable for large scale extraction of DNA from fresh or frozen animal tissues or cells.
- **ü** Economic.

Materials Supplied by User:

Microcentrifuge capable of at least 12,000 × *g*
Pipets and pipet tips
Vortexer
Ethanol (96-100%)
RNase A (100 mg/ml, Optional for RNA-free DNA)
Microcentrifuge tubes (1.5 ml or 2 ml)
Water bath for heating at 56°C

Before Starting

- **Ø** Proteinase K is supplied in a ready-to-use solution form, but RNase A is not provided in this kit, if RNA-free DNA is required, please prepare RNA solution and follow steps for RNA removal.
- **Ø** 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.
- **Ø** 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.
- **Ø** **CW1 Solution** and **CW2 Solution** are supplied as concentrates. Add ethanol (96-100%) to CW1 Solution and CW2 Solution before use to obtain a working solution (Volume of ethanol:Volume of CW1 Solution=17:13; Volume of ethanol:Volume of CW2 Solution=7:3).
- **Ø** Preheat the water bath or rocking platform to 56°C.

Procedures

1. Sample Preparation.

A. Non-nucleated: Add 1 ml proteinase K into a 50 ml centrifuge tube. Add 3-5 ml anticoagulated blood. Adjust the volume to 11 ml with PBS. Proceed with step 2.

B. Nucleated: add 1 ml proteinase K into a 50 ml centrifuge tube; add 300-500 µl anticoagulated blood.

Adjust the volume to 11 ml with PBS. Proceed with step 2.

Note: If RNA-free genomic DNA is required, add 4 µl RNase A (20 mg/ml). Mix by vortexing, and incubate for 2 min at room temperature before continuing with step 2.

2. Add 10 ml Buffer CL to the sample. Mix thoroughly by vortexing. Incubate at 56°C for 20 minutes.

3. Add 10 ml ethanol (96-100%), mix thoroughly by vortexing.

4. Transfer the mixture from step 3 (including any precipitate) into the EZ-500 spin column placed in a 50 ml collection tube. Centrifuge at 9,000 *x g* (12,000 *rpm*) for 1 minute. Discard the flow-through.

Note: If the column cannot hold entire supernatant. Please apply in multiple times. Spin briefly (1-2 minutes) at 4,000 x g between each application. After the final application of entire content in the column, spin at 4,000 x g for 3-5 minutes.

5. Add 5 ml CW1 Solution, and centrifuge for 1 minute at 9,000 *x g* (12,000 *rpm*). Discard the flow-through.

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

6. Add 5 ml CW2 Solution, and centrifuge for 1 minute at 9,000 *x g* (12,000 *rpm*). Discard the flow-through.

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

7. Place the empty spin column in the 50 ml collection tube and centrifuge for an additional 2 minutes at 9,000 *x g* (12,000 *rpm*) to dry the EZ-500 membrane. Discard flow-through and transfer the spin column to a clean 50 ml centrifuge tube.

Note: It is important to dry the membrane of the EZ-500 spin column, 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 1 ml Buffer CE directly onto the center part of EZ-500 membrane. Incubate at room temperature for 1 minute, and then centrifuge for 1 minute at 9,000 *x g* (12,000 *rpm*) to elute the DNA.

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

Note 2: 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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