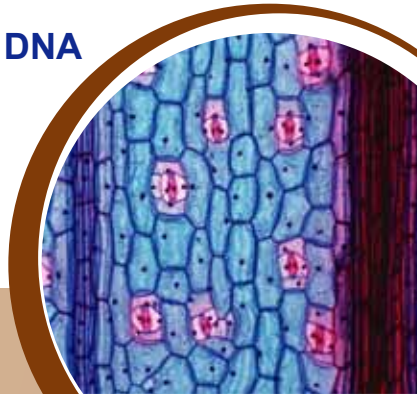
BIO BASIC INC.

EZ-10 SPIN COLUMN GENOMIC DNA MINIPREPS KIT HANDBOOK

(Bacteria, Plant, Animal, Blood)



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BIO BASIC INC.

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

ISO9001 Certified

www.biobasic.com

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Introduction

EZ-10 Spin Column Kits provide a fast, simple and efficient method for purification of genomic DNA from various sources such as Bacteria, Plant tissue, Animal tissue, Cells and Blood.

By taking the advantage of our silica-based DNA purification technology, DNA is selectively adsorbed in the silica-based membrane embedded in EZ-10 Spin Column. Other components and impurities flow through the column or are washed away during wash steps. Genomic DNA is then eluted off the column and can be readily used in most downstream applications, including restriction enzyme digestion, PCR, Southern-blotting, etc.

The purification procedure using in these kits does not require use of hazardous compounds such as phenol, chloroform, or CsCl. DNA is purified without additional steps of ethanol precipitation.

Limitations of Use

These kits are designed for research use only. Purified DNA should not be used for live animal transfections. It is also not to be used for human diagnostic or drug production purposes.

Features

√ Simple, fast and efficient.
√ Preparation of high quality genomic DNA from various sources.
√ High yield and reproducible.
√ No phenol chloroform extraction or ethanol precipitation required.
√ High capacity - up to 10 µg of DNA per column.

Applications

Purification of up to 10 µg genomic DNA from various sources.

Storage

All components (except Proteinase K) can be stored at room temperature. Proteinase K should be kept at 4ºC for short-term or -20ºC for long-term storage. Kit components are stable for 12 months at room temperature after received. For maximum stability, store all contents at 4ºC.

Quality Control

Each lot of EZ-10 Spin Column kit is tested against predetermined specifications to ensure consistent product quality.

EZ-10 Spin Column Genomic DNA Minipreps Kit, Bacteria

Kit Contents

Component	BS423, 50 Preps	BS624, 250 Preps
Digestion Solution[(a)]	20 ml	100 ml
Wash Solution[(b)]	12 ml	2 x 30 ml
Elution Buffer[(c)]	5 ml	25 ml
Proteinase K[(d)]	2 mg	10 mg
EZ-10 Column (with 2.0-ml Collection Tube)	50	250
Protocol	1	1

Notes:

a. Digestion Solution may form a precipitate upon storage. Dissolve the precipitate by warming the solution to 37ºC if necessary.
b. Before use, add 48 ml of 100% ethanol to 12 ml Wash Solution for BS423, or 120 ml of 100% ethanol to 30 ml Wash Solution for BS624. For other volumes of Wash Solution, simply add ethanol to make a 4:1 ratio (volume of added ethanol:volume of Wash Solution = 4:1).
c. The Elution Buffer composition is 2.0 mM Tris-HCl, pH 8.0 - 8.5. Water can also be used but yield may be slightly lower.
d. Before use, add 150 µl (or 750 µl) of sterilized water to the tube containing 2 mg (or 10 mg) of proteinase K, respectively. For long term storage, proteinase K solution should be kept at -20ºC.

Principle

This kit is designed for rapid isolation of genomic DNA from cells and bacteria. The kit contains a membrane embedded column for binding up to 10 µg of genomic DNA. Nucleotides, proteins, salts, and other impurities are washed away. Purified genomic DNA can be used in most molecular biology experiments including restriction enzyme digestion, PCR, Southern-blotting, etc.

Procedures for Isolation of Genomic DNA from Cells or Bacteria

1. Sample Preparation.

A. Cell Cultures

(1) Cells grown in suspension

Spin desired number of cells (not exceeding 5 x 10^6 cells) at 2,500 *x g* (5,000 *rpm*) for 5 minutes at room temperature. Remove supernatant completely. Wash the cell pellet twice with PBS Buffer (not provided with kit) and resuspend cells in 200 µl cold TE Buffer (not provided with kit), proceed to Step 2.

(2) Cells grown in monolayer

Aspirate the medium and wash cells with PBS Buffer. Remove PBS and apply trypsin solution to the cells. After cells have become detached, neutralize the trypsin with 2 volumes of medium. Centrifuge at 8,000 x g (10,000 *rpm*) for 5 minutes. Carefully remove supernatant and resuspend pellet in 200 µl TE buffer, and continue with Step 2.

√	If following steps cannot be performed immediately, it is safe to pause here and it is recommended to store the lysate at -20 ºC or -80 ºC.
√	Avoid repeated freezing and thawing of stored samples, since this leads to reduced DNA size and yield.

B. Bacteria Collection

Spin appropriate number of bacteria (about 10^6~10^7) at 6,000 *x g* (8,000 *rpm*) for 5 minutes at room temperature. Remove supernatant completely and resuspend cells in 200 µl cold TE (not provided with kit), proceed to Step 2.

C. Paraffin Tissue

(1) Excise 25~30 mg paraffin tissue with a clean, sharp scalpel. Transfer to a 1.5 ml Eppendorf tube.
(2) Add 1.2 ml xylene (not provided with kit, Xylene is used to remove paraffin) to the tube, vortex for 3 minutes.
(3) Centrifuge at 10,000 *x g* (12,000 *rpm*) for 5 minutes at room temperature.
(4) Discard the supernatant and keep the pellet.
(5) Add 1.2 ml of 100% ethanol to the tube. Gently vortex for 1 minute. Incubate at room temperature for 1 minute.
(6) Centrifuge at 10,000 *x g* (12,000 *rpm*) for 5 minutes at room temperature. Discard supernatant.
(7) Repeat step 5 and 6.
(8) Incubate at 37 ºC for 10-15 minutes to remove residual ethanol.
(9) Resuspend the sample in 200 µl TE buffer, and proceed to Step 2.

2. Add 400 µl of Digestion Solution to 200 µl sample from step 1. Mix well. Add 3 µl of Proteinase K solution (2mg/150 µl) to sample and incubate at 55 ºC for 5 minutes.

√	Do not add proteinase K solution directly to Digestion Solution.
√	Incubation period depends on the nature of sample. For cell cultures, 5 minutes is generally enough to obtain complete lysate. Tissue samples may require 3-5 hours. Longer periods of incubation (even overnight) will not affect the result.
√	If RNA-free genomic DNA is required, add 20 µl RNase A (10 mg/ml, not provided with kit), mix by vortexing, and incubate for 5 min at room temperature before continuing with step 3.

3. Add 260 µl of 100% ethanol, and mix well. Apply the mixture onto an EZ-10 spin column that is placed in a 2.0 ml Collection Tube. Spin at 8,000 *x g* (10,000 *rpm*) for 2 minutes.

4. Discard the flow-through in the collection tube. Add 500 µl of Wash Solution, and spin at 8,000 *x g* (10,000 *rpm*) for 2 minutes.

5. Repeat Step 4.

6. Discard flow-through. Spin at 8,000 *x g* (10,000 *rpm*) for an additional minute to remove residual amount of Wash Solution.

7. Place the EZ-10 column into a clean 1.5 ml Eppendorf tube. Add 30-50 µl Elution Buffer into the center part of membrane in the column. Incubate at RT for 2 or 3 minutes. Incubating the tube at 37ºC or 50ºC for 2 minutes may increase recovery yield.

8. Spin at 8,000 *x g* (10,000 *rpm*) for 2 minute to elute DNA from the column.

9. For long term storage, keep aliquots of purified genomic DNA at -20 ºC.

10. Measure DNA quantity by UV absorption at A260 (1.0 OD unit is equivalent of 50 µg). Assess genomic DNA quality by an analytical 0.7% agarose gel. The length of genomic DNA is around 50 kb.

Troubleshooting Guide: EZ-10 Spin Column Genomic DNA Minipreps Kit, Bacteria

1. Low yield

a. Improper storage of starting material
>>>Prepare fresh samples and use immediately.
b. Too much or too little starting material
>>>Reduce or increase starting material accordingly.
c. Incorrect preparation of buffers
>>>Each step has to be strictly followed.

2. RNA contamination

Perform optional RNase treatment according to the protocol.

3. $OD_{260nm/280nm}$ ratio outside 1.6-2.2 range

If the ratio of OD260nm/280nm is greater than 2.2, there may be traces of ethanol present. If the ratio of OD260nm/280nm is smaller than 1.6, there may be protein contamination. Make sure the sample is mixed well after proteinase K digestion.

4. DNA does not perform well

a. DNA Shearing
>>>Avoid repeated freezing and thawing of starting material; if samples are too old, start with a new sample.
b. Ethanol Carryover
>>>Spin additional steps before elution.

EZ-10 Column Plant Genomic DNA Purification Kit

Kit Contents:

Components	SK8262, 100 Preps
Buffer PCB	80 ml
Buffer BD	60 ml
PW Solution	36 ml
Wash Solution	15 ml
TE Buffer (pH 8.0)	20 ml
EZ-10 Column & Collection Tube	100
Protocol	1

Storage

EZ-10 columns and all buffers should be stored dry, at room temperature (15-25°C). The kit is stable for 1 year under these conditions.

Safety Instructions

Buffer PCB and Buffer BD are harmful in contact with skin if swallowed, please avoid contact with eyes, skin, and clothes. Wash thoroughly after handling and see a doctor if necessary.

Introduction

The kit provides a simple and convenient technique to isolate high quality DNA from plants using a rapid spin-column format. DNA of cell lysates is selectively bound to the spin column and other impurities such as proteins and salts do not bind to the column and are eliminated in flow through. No phenol extraction and ethanol precipitations are required. Purified genomic DNA is 20-50 kb in length. Purified DNA is suitable for downstream applications such as Restriction Endonuclease Digestions, PCR, and other applications.

Features

√ Fast and easy. Processing uses a rapid spin-column format. The entire procedure takes approx. 30 minutes.

√ Versatile. Various plant species have been tested.
√ High quality of DNA. OD_{260}/OD_{280} of purified DNA is generally 1.7-1.9. The purified DNA is ready-to-use for most downstream applications.

Materials Supplied by User

Microcentrifuge capable of at least 12,000 × *g*
Pipette tips
Vortexer
Isopropanol
β-mercaptoethanol
Ethanol (96-100%)
RNase A (20 mg/ml, Optional for RNA-free DNA)
Microcentrifuge tubes (1.5 ml or 2 ml)
Water bath for heating at 65°C

Before Starting

This protocol is designed for purification of total DNA from plant. All centrifugation steps are carried out at room temperature (15-25°C) in a microcentrifuge. It is strongly advised that you read this protocol thoroughly before starting. The EZ-10 Column Plant 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.

Check the Buffer PCB and Buffer BD for salt precipitation before each use. If necessary, re-dissolve the precipitate by warming the solution at 65°C, then cool back down to room temperature before use.

TE Buffer is 10 mM Tris-HCl, 1 mM EDTA, pH 8.0. Water can be used as elutant in the final step if EDTA must be avoided for downstream applications, but it is not recommended if the pH of water is less than 7.0.

PW Solution and Wash Solution are supplied as concentrates. Before using for the first time, add 24 ml isopropanol to 36 ml PW Solution, and 45ml ethanol to 15ml Wash Solution, respectively. Preheat the water bath or rocking platform to 65°C.

Procedure:

1. Grind 100 mg fresh plant tissue (or 20 mg dry plant tissue) to fine powder in liquid nitrogen. Transfer the powder to a 1.5 ml tube.

2. Add 600 µl Buffer PCB and 12 µl of β-mercaptoethanol to the sample, and mix thoroughly by vortexing. Incubate at 65°C for 25 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 0.6 ml of chloroform to the tube, mix well by inverting 10 times. Centrifuge at 12,000 x *g* for 2 minutes. Carefully transfer the supernatant (400 µl) to a clean 1.5 ml tube.

4. Add 200 µl Buffer BD, mix thoroughly by vortexing.
   Note: If a gelatinous material appears at this step, incubate at 70°C for 10 min.

5. Add 200 µl ethanol (96-100%), mix thoroughly by vortexing.
   Note: If a gelatinous material appears at this step, vigorously shaking or vortexing is recommended.

6. Transfer the mixture from step 5 (including any precipitate) into the EZ-10 column placed in a 2 ml collection tube. Centrifuge at 9,000 x *g* (12,000 *rpm*) for 1 min. Discard the flow-through.

7. Add 500 µl PW Solution, and centrifuge for 1 min at 9,000 x *g* (12,000 *rpm*). Discard the flow-through.
   Note: Check the label to ensure PW Solution was diluted with isopropanol.

8. Add 500 µl Wash Solution, and centrifuge for 1 min at 9,000 x *g* (12,000 *rpm*). Discard the flow-through.
   Note: Check the label to ensure Wash Solution was diluted with ethanol.

9. Place the empty column in the microcentrifuge and centrifuge for an additional 2 min at 9,000 x *g* (12,000 *rpm*) to dry the EZ-10 membrane. Discard flow-through and transfer the spin column to a clean 1.5 ml centrifuge tube.
   Note: It is important to dry the membrane of the EZ-10 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.

10. Add 50-100 µl TE Buffer directly onto the center part of EZ-10 membrane.

Incubate at room temperature for 1 min, and then centrifuge for 1 min at 9,000 x *g* (12,000 *rpm*) to elute the DNA.
Note 1: Warming the TE Buffer to 60°C will increase the elution efficiency.
Note 2: For maximum DNA yield, repeat elution once as described in this step.
Note 3: A new microcentrifuge tube can be used for the second elution step to prevent dilution of the first eluate.
Note 4: To maximize DNA concen tration, use the eluate in the microcentrifuge tube for the second elution step.

EZ-10 Spin Column Genomic DNA Minipreps Kit, Animal

Kit Contents

Component	BS427, 50 Preps	BS628, 250 Preps
ACL Solution[(a)]	20 ml	100 ml
PBS Solution	75 ml	2 x 200 ml
AB Solution	20 ml	100 ml
Proteinase K[(b)]	20 mg	100 mg
Wash Solution[(c)]	12 ml	2 x 30 ml
Elution Buffer[(d)]	5 ml	25 ml
EZ-10 Column (with 2.0-ml Collection Tube)	50	250
Protocol	1	1

Note:

a. ACL Solution may form a precipitate upon storage. If necessary, dissolve the precipitate by warming the solution to 37ºC.
b. Before use, add 1 ml (or 5 ml) of sterilized water to the tube containing 20 mg (or 100 mg) of Proteinase K. Keep solution at -20ºC.
c. Before use, add 48 ml of 100% ethanol to 12 ml Wash Solution for BS427 or 120 ml of 100% ethanol to 30 ml Wash Solution for BS628. For other volumes of wash solution, simply add enough ethanol to make a 4:1 ratio (volume of added ethanol:volume of Wash Solution = 4:1).
d. Elution Buffer is 2.0 mM Tris-HCl pH 8.0~8.5. Although TE buffer pH 8.0 or water can be used, yield is generally 10% lower.

Procedure for Isolation of Genomic DNA from Animal

For Animal Tissue

1. Cut up to 30 mg of tissue and place in a 1.5 ml centrifuge tube.

2. Add 300 µl of ACL Solution (Animal Cell Lysis Solution) to 1.5 ml centrifuge tube and 20 µl of Proteinase K.

3. Incubate at 55ºC until tissues are completely lysed (usually 1-3 hours). Vortex occasionally. Incubating the sample in a shaking water bath can reduce lysis time.

> √ If RNA-free genomic DNA is required, add 20 µl RNase A (10 mg/ml, not provided with kit), mix by vortexing, and incubate for 5 min at room temperature before continuing with step 5.

4. Cool to room temperature. Vortex for 20 seconds and centrifuge 10,000 *x g* (12,000 *rpm*) for 5 minutes.

5. Pipette 300 µl of supernatant to a new Eppendorf tube, add 300 µl of AB Solution. Mix by occasionally inverting tube, and keep for 2 minutes. Then load all the solution into an EZ-10 Spin Column.

6. Centrifuge at 2,000 *x g* (4,000 *rpm*) for 2 minutes and discard the flow-through.

7. Add 500 µl of Wash Solution, and spin at 8,000 *x g* (10,000 *rpm*) for 2 minutes.

8. Repeat Step 8.

9. Discard flow-through. Spin at 8,000 *x g* (10,000 *rpm*) for an additional minute to remove residual amount of Wash Solution.

10. Place the column into a clean 1.5 ml Eppendorf tube. Add 30-50 µl Elution Buffer into the center part of membrane in the column. Incubate at RT for 2 or 3 minutes. Incubating the tube at 37ºC or 50ºC for 2 minutes may increase recovery yield.

11. Spin at 8,000 *x g* (10,000 *rpm*) for 1 minute to elute DNA from the column.

12. For long term storage, keep aliquots of purified genomic DNA at -20 ºC.

13. Measure DNA quantity by UV absorption at A_{260} (1.0 OD unit is equivalent of 50 ug). Assess genomic DNA quality by an analytical 0.7% agarose gel.

For Rodent Tail

1. Place numbered 1.5 ml centrifuge tubes on dry ice.

2. Cut 0.5 cm to 1 cm from ends of tails and place in tubes.

3. Add 300 µl of ACL Solution to 1.5 ml centrifuge tubes and then add 20 µl of Proteinase K.

4. Incubate at 55ºC overnight with rocking; or for several hours with occasional mild vortexing every 15 minutes.

> √ If RNA-free genomic DNA is required, add 20 µl RNase A (10 mg/ml, not provided with kit), mix by vortexing, and incubate for 5 min at room temperature before continuing with step 6.

5. Cool to room temperature. Vortex for 20 seconds and centrifuge at 10,000 *x g* (12,000 *rpm*) for 5 minutes.

6. Pipette 300 µl of supernatant into to a new Eppendorf tube, add 300 µl of AB Solution. Mix by occasionally inverting tube, and keep for 2 minutes. Then load all the solution to a EZ-10 Spin Column.

7. Centrifuge 2,000 *x g* (4,000 *rpm*) for 2 minutes and discard the flow-through.

8. Add 500 µl of Wash Solution, and spin at 8,000 *x g* (10,000 *rpm*) for 1 minute.

9. Repeat Step 9.

10. Discard flow-through. Spin at 8,000 *x g* (10,000 *rpm*) for an additional minute to remove residual amount of Wash Solution.

11. Place the column into a clean 1.5 ml Eppendorf tube. Add 30-50 µl Elution Buffer into the center part of membrane in the column. Incubate at RT for 2

or 3 minutes. Incubating the tube at 37ºC or 50ºC for 2 minutes may increase recovery yield.

12. Spin at 8,000 *x g* (10,000 *rpm*) for 1 minute to elute DNA from the column.

13. For long term storage, keep aliquots of purified genomic DNA at -20ºC.

14. Measure DNA quantity by UV absorption at A_{260} (1.0 OD unit is equivalent of 50 µg). Assess genomic DNA quality *by* an analyt*ica*l 0.7% agarose gel.

For Cultured Animal Cell

1. Centrifuge the appropriate number of cells (>5x10^6) for 5 minutes at 200 *x g* (1,200 *rpm*).

2. Resuspend pellet in 500 µl of PBS Solution.

3. Wash the cells 2 times with PBS Solution.

4. Resuspend pellet in 300 µl of ACL solution buffer.

5. Add 20 µl of Proteinase K, mix well and Incubate at 55 ºC for 10 minutes.

> √ If RNA-free genomic DNA is required, add 20 µl RNase A (10 mg/ml, not provided with kit), mix by vortexing, and incubate for 5 min at room temperature before continuing with step 7.

6. Cool to room temperature. Vortex for 20 seconds and centrifuge 10,000 *x g* (12,000 *rpm*) for 5 minutes.

7. Pipette 200 µl of supernatant to a new Eppendorf tube, add 200 µl of AB Solution. Mix by occasionally inverting tube, and keep for 2 minutes. Then load all the solution to a EZ-10 Spin Column.

8. Centrifuge at 2,000 *x g* (4,000 *rpm*) for 2 minutes and discard the flow-through.

9. Add 500 µl of Wash Solution, and spin at 8,000 *x g* (10,000 *rpm*) for 1 minute.

10. Repeat Step 10.

11. Discard flow-through. Spin at 8,000 *x g* (10,000 *rpm*) for an additional minute to remove residual amount of Wash Solution.

12. Place the column into a clean 1.5 ml Eppendorf tube. Add 30-50 µl Elution Buffer into the center part of membrane in the column. Incubate at RT for 2 or 3 minutes. Incubating the tube at 37ºC or 50ºC for 2 minutes may increase recovery yield.

13. Spin at 8,000 *x g* (10,000 *rpm*) for 2 minutes to elute DNA from the column.

14. For long term storage, keep aliquots of purified genomic DNA at -20 ºC.

15. Measure DNA quantity by UV absorption at A_{260} (1.0 OD unit is equivalent of 50 ug). Assess genomic DNA quality by an analytical 0.7% agarose gel.

From Paraffin Tissue

1. Excise 25~30 mg paraffin tissue with a clean, sharp scalpel and transfer to a 1.5 ml Eppendorf tube.

2. Add 1.2 ml xylene (not included in the kit) to the tube, then vortex for 3 minutes. Xylene is used to remove paraffin.

3. Centrifuge at 10,000 *x g* (12,000 *rpm*) for 5 minute at room temperature.
4. Discard the supernatant and keep the pellet.

5. Add 1.2 ml 100% of ethanol to the tube. Gently vortex for 1 minute. Incubate at room temperature for 1 minute.

6. Centrifuge at 10,000 *x g* (12,000 *rpm*) for 5 minute at room temperature. Discard supernatant.

7. Repeat steps 4 to 6.

8. Incubate at 37 ºC for 10-15 minutes to remove residual ethanol.

9. Resuspend the sample in 200 µl TE buffer, and proceed immediately to Step 10.

10. Add 300 µl of ACL Solution (Animal Cell Lysis Solution) and then add 20 µl of Proteinase K.

√	If RNA-free genomic DNA is required, add 20 µl RNase A (10 mg/ml, not provided with kit), mix by vortexing, and incubate for 5 min at room temperature before continuing with step 12.

11. Incubate at 55 ºC until the tissue is completely lysed (usually 1-3 hours). Vortex occasionally. Incubation in shaking water bath can reduce lysis time.

12. Cool to room temperature. Vortex for 20 seconds and centrifuge at 10,000 *x g* (12,000 *rpm*) for 5 minutes.

13. Pipette 300 µl of supernatant to a new Eppendorf tube, add 300 µl of AB Solution. Mix by occasionally inverting tube, and keep for 2 minutes. Then load all the solution to a EZ-10 Spin Column.

14. Centrifuge at 2,000 *x g* (4,000 *rpm*) for 2 minutes and discard the flow-through.

15. Add 500 µl of Wash Solution, and spin at 6,000 *x g* (8,000 *rpm*) for 1 minute.

16. Repeat Step 15.

17. Discard the flow-through and spin at 8,000 *x g* (10,000 *rpm*) for an additional minute to remove residual amount of Wash Solution.

18. Place the column into a clean 1.5 ml Eppendorf tube. Add 30-50 µl Elution Buffer into the center part of membrane in the column. Incubate at RT for 2 or 3 minutes. Incubating the tube at 37ºC or 50ºC for 2 minutes may increase recovery yield.

19. Spin at 8,000 *x g* (10,000 *rpm*) for 1 minute to elute DNA from the column.

20. For long term storage, keep aliquots of purified genomic DNA at -20 ºC.

21. Measure DNA quantity by UV absorption at A_{260} (1.0 OD unit is equivalent of 50 µg). Assess genomic DNA quality by an analytical 0.7% agarose gel.

EZ-10 Spin Column Genomic DNA Minipreps Kit, Blood (New!)

Kit Contents

Component	SK8253, 50 Preps	SK8254, 100 Preps
PBS Solution	8 ml	16 ml
Buffer CL[(a)]	12 ml	24 ml
CW1 Solution(Concentrate) [(b)]	13 ml	26 ml
CW2 Solution(Concentrate) [(b)]	9 ml	18 ml
CE Buffer (pH 9.0)	15 ml	30 ml
Buffer TBP (optional)	50 ml	100 ml
Proteinase K[(c)]	1.2 ml	2.4 ml
EZ-10 Column (with 2.0-ml Collection Tube)	50	100
Protocol	1	1

Note:

a. Buffer CL Solution may form a precipitate upon storage. If necessary, dissolve the precipitate by warming the solution to 56ºC.
b. Before use, add 17 ml of 100% ethanol to 13 ml CW1 Solution (Concentrate) and 21 ml of 100% ethanol to 9 ml of CW2 Solution (Concentrate) for SK8253; add 34 ml of 100% ethanol to 26 ml CW1 Solution (Concentrate) and 42 ml of 100% ethanol to 18 ml of CW2 Solution (Concentrate) for SK8254.
c. Proteinase K is supplied in liquid and is stable at Room temperature. For long term storage, please keep at -20ºC.
d. CE Buffer is 10 mM Tris-HCl, 0.5 mM EDTA, pH 9.0. Although TE buffer pH 8.0 or water can be used, yield is generally 20% lower.

Storage of Blood

Whole blood samples treated with EDTA, ACD or heparin can be used, and may be either fresh or frozen. For short term storage (up to 10 days), collect blood in tubes containing EDTA as an anticoagulant, and store tubes at 2-8ºC. It is

recommended to store blood samples less than 3 days as DNA degradation may occur. For long term storage, collect blood in tubes containing a standard anticoagulant (preferably EDTA if high molecular weight DNA is required) and store at -80ºC.

Blood Collection and Treatment

For every 1 ml of whole blood sample, add 0.1 ml of anticoagulant (0.5M EDTA pH 8.0, or ACD, 0.48% Citric Acid, 1.32% Sodium Citrate, 1.47% Glucose).

Procedure for Extraction Genomic DNA from Blood

1. Sample Preparation

A. Blood Samples (non-nucleated Erythrocytes, for example Human Blood): Collect ~100ul of blood into 2.0 ml centrifuge tube. Add PBS solution to the tube to a final volume of 200ul. Vortex gently and let the tube stand for 1 min at room temperature.

> √ If >100ul of blood is used, add 2 volumes of Buffer TBP. Mix thoroughly and let the tube stand for 1 min until red cells lyse completely. Spin at 4,000 Xg (8,000 rpm) for 1 min. Discard the supernatant carefully. Wash the precipitate with 500ul TE Buffer 2 times. Spin at 4,000 Xg (8,000 rpm) for 1 min during each wash. The final precipitate should appear white. Proceed with step 2.
> √ Typical Yield is 1-3ug from 100ul blood sample.

B. Blood Samples (Nucleus-containing Erythrocytes, for example chicken Blood): Collect ~10ul of blood into a 2.0 ml centrifuge tube. Add PBS solution to the tube to a final volume of 200ul. Vortex gently and let the tube stand for 1 min at room temperature. Proceed to step 2.

C. Solidified Blood Clot: Weigh 0.1g of blood. Grind to find powder under liquid nitrogen. Add 200ul of PBS solution and proceed to step 2.

2. Add 20ul of proteinase K. Mix well. Add 200 ul of Buffer CL. Vortex Gently. Incubate at 56ºC for 10min.

> √ The solution should appear clear after complete lysis. If solution still appears cloudy, please extend incubation time until lysis is complete and solution is clear.
> √ If RNA-free genomic DNA is required, add 20 µl RNase A (10 mg/ml, not provided with kit), mix by vortexing, and incubate for 5 min at room temperature before continuing with step 3.
> √ If final reaction volume is more than 500ul, please increase proteinase K usage and/or extend incubation time.

3. Add 200ul of 100% ethanol to the mixture and mix thoroughly.

> √ Small cloudy insoluble material may appear after addition of ethanol, but this does not affect the performance of the kit. Proceed with step 4.

4. Transfer the mixture from step 3 (including any precipitates) into an EZ-10 column that is in a 2.0 ml Collection Tube. Let it stand at Room Temperature for 1-2min. Spin at 8,000 *x g* (10,000 *rpm*) for 2 minutes. Discard the flow-through in the collection tube.

5. Add 500 µl of CW1 Solution, and spin at 8,000 *x g* (10,000 *rpm*) for 1 minute.

> √ Please ensure ethanol has been added to the CW1 concentrate prior to usage

6. Add 500 µl of CW2 Solution, and spin at 8,000 *x g* (10,000 *rpm*) for 1 minute.

> √ Please ensure ethanol has been added to the CW2 concentrate prior to usage

7. Discard the flow-through. Spin at 8,000 *x g* (10,000 *rpm*) for an additional minute to remove any residual amount of CW2 Solution.

8. Place the column into a clean 1.5 ml Eppendorf tube. Add 30-50 µl CE Buffer into the center part of membrane in the column. Incubate at RT for 2 to 3 minutes. Incubating the tube at 37ºC or 50ºC for 2 minutes may increase recovery yield.

9. Spin at 8,000 *x g* (10,000 *rpm*) for 1 minute to elute DNA from the column.

10. For long term storage, keep aliquots of purified genomic DNA at -20 ºC. Measure DNA quantity by UV absorption at A_{260} (1.0 OD unit is equivalent of 50 µg). Assess genomic DNA quality by an analytical 0.7% agarose gel.

Troubleshooting Guide: EZ-10 Spin Column Genomic DNA Minipreps Kit, Blood

Low yield

There are a number of variables that can cause low yield.
a. Each step has to be strictly followed.
b. Make sure column binding capacity of 10 µg is not exceeded.

RNA contamination

RNase activity is weakened or lost. Add 30% additional RNase A, and store solution at 4ºC.

Sample floats upon loading in agarose gel

The sample contains ethanol from washing step. Discard the liquid waste from the collection tube after washing step, and spin again for additional two minutes. Before elution step, incubate the column at 50ºC for ~5 min and allow ethanol to evaporate completely.

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