Introduction

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

DNA is selecti ely adsorbed in the silica-based membrane embedded in EZ-10 Spin Column. Other components and impuritiesflow through the column or are washed away during wash steps. Genomic DNA is then eluted o ffthe column and can be readily used in most downstream applications,including resstrictioenzyme digesestioPCR, R, Southern-blo , etc.

The purificaatioprocedure using in these kits does not require use of hazardous compounds such as phenol, chloroform, or CsCl. DNA is purified without additionasteps 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 diagnosstior drug prproduction purposes.

Features

- Simple, fast and efficient.
- Preparationof high quality genomic DNA from various sources.
- No phenol chloroform extractionor ethanol precipitaatiorequired.
- High capacity - up to 10 µg of DNA per column.

Applications

Purificaatioof up to 10 µg genomic DNA from various sources.

Storage Conditions

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

Quality Control

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

EZ-10 Spin Column Bacterial Genomic DNA Mini-Preps Kit

Kit Contents

Component	BS423, 50 Preps	BS624, 250 Preps
EZ-10 Column	50	250
2.0 ml Collection ube	50	250
Universal Buffer Digesstion	10 ml	50 ml
Universal Buffer BD	12 ml	60 ml
Universal PW Solution (oncentrate)	18 ml	90 ml
Universal Wash Solution (oncentrate)	7.5 ml	37.5 ml
CE Buffer	15 ml	75 ml
Proteinase K	1.2 ml	6 ml
Protocol	1	1

NOTE 1: Universal Buffer BD contains a chaotropic salt, avoid contact with skin and eyes.
NOTE 2: Universal PW Solution and Uni ersal WWash Solution e supplied as concentrates. Add 12 ml isopropanol to 18 ml Universal PW Solutionand 22.5 ml ethanol (96-100%) for 7.5 ml Universal Wash Solutionbefore use to obtain a working solution.

Storage and stability

Ezup columns and all buffers should be stored dry, at room temperature (15-25°C) and are stable for 1 year under these conditons. Proteinase K is supplied as 10 mg/ml soluuon, the soluton can be keep at Room Temperature for 6 months, for long-term storage keep at -20°C.

Introduction

The kit provides a simple and convenient technique to isolate high quality DNA from both Gram negati e and Gram posit e bacteria using a rapid spin-column format. DNA of cell lysate is selecti ely bound to the spin column and other impuritiesuch as proteins, salts do not bind on the column and are eliminated in flow through. No phenol extrractionno ethanol precipitationsare required. The kit is also suitable for isolaatioof bacterial genomic DNA from colonies on dish. Purified genomic DNA can be up to 50 kb in length. Purified DNA is suitable for downstream applicationssuch as ResstrictioEndonuclease DigesestionPCR, and other applications.

Features

- Fast and easy processing using a rapid spin-column format. The entie procedure takes approximately 30 minutes.
- High yield. 5-20 µg of bacterial genomic DNA can be obtained from 1 ml (10^8-10^9 cells) of overnight culture.
- High quality of DNA. $OD_{260}/_{280}$ of purified DNA is generally 1.7-1.9.
- No phenol/chloroform extractionor ethanol precipitaatiois required.

Materials Supplied by User

- Microcentrifuge capable of at least 12,000 × g
- Pipetes and pipeee e tips
- Vortexer
- Isopropanol
- Ethanol (96-100%)
- Lysozyme (for Gram positi e bacteria)
- Rnase A (20 mg/ml, Optionalfor RNA-free DNA)
- Microcentrifuge tubes (1.5 ml or 2 ml)
- Water bath for heatingat 56°C

Before Starting

This protocol is designed for purificaatioof total DNA from Gram m posie or Gram negaege bacteria. All centrifugation steps are carried out at room temperature (15-25°C) in a microcentrifuge. It is strongly advised that you read this protocol throughly before starting. Ezup Column Bacteria Genomic DNA PurificaatioKit 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 solutionform, but RNase A is not provided in this kit, if RNA-free DNA are required, please prepare RNA solutionand see protocol to add the RNA removal step.

For Gram Positi e bacteria, cell wall should be removed by an enzyme (e.g. Lysozyme) before lysis, but the enzyme is NOT supplied in the kit.

Check the Universal Buffer Digesstioand Universal Buffer BD for salt precipitaitat before each use. If necessary, redissolve the precipitate by warming the solutionat 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.

Universal PW Solutionand Universal Wash Solutio are supplied as concentrates. Before using for the first timeadd 12 ml isopropanol to 18 ml Universal PW W Solutio22.5 ml ethanol to 7.5 ml Universal Wash Solution,rrespectely.

Preheat the water bath or rocking platorm to 56°C.

Protocol

1. Sample Preparation
   - **A.** Gram-negati e bacterials (E. coli, streptococcal, pneumococcal, etc.)
     Transfer overnight culture (about 2 x 10^9 cells) into centrifuge tube and centrifuge at 10000 x g for 30 seconds, discard supernatant.
   - **B.** Gram-positi e bacterials (golden staphylococcal, orynebacteriadiphtheriae, etc.)
     a) Transfer overnight culture (about 2 x 10^9 cells) into centrifuge tube and centrifuge at 10000 x g for 30 seconds, discard supernatant.

b) Add 180 µl lysozyme solution(20 mg/ml lysozyme, 20 mM Tris-HCl, pH 8.0, 2.5 mM EDTA, 1% Triton X-100. NOT supplied in the kit), suspend thoroughly and incubate at 37°C for 30-60 minutes. Add 20 µl Proteinase K and mix thoroughly by vortexing. Incubate at 56°C for 30 min. Continuingwith step 3.

2. Add 180 µl Universal Buffer Digesstio and 20 µl Proteinase K to the sample, and mix thoroughly by vortexing. Incubate at 56°C for 1 hour.

   NOTE: If RNA-free genomic DNA is required, add 20 µl RNase A (20 mg/ml), mix by vortexing, and incubate for 2 minutes at room temperature then continuewith step 3.

3. Add 200 Universal Buffer BD, mix thoroughly by vortexing. Incubate at 70°C for 10 minutes.
4. Add 200 µl ethanol (96-100%), mix thoroughly by vortexing.

   NOTE: If a gelatinousmaterial appears at this step, vigorously shaking or vortexing is recommended.

5. Transfer the mixture from step 4 (including any precipitate) into the Ezup column placed in a 2 ml collectiontube. Centrifuge at 9,000 x g (12,000 rpm) for 1 minute. Discard the flow-through.
6. Add 500 µl Universal PW Solution,and centrifuge for 1 minute at 9,000 x g (12,000 rpm). Discard the flow-through.

   NOTE: Check the label to ensure Universal PW Solutionwas diluted with isopropanol.

7. Add 500 µl Universal Wash Solution,and centrifuge for 1 minute at 9,000 x g (12,000 rpm). Discard the flow-through.

   NOTE: Check the label to ensure Universal Wash Solutionwas diluted with ethanol.

8. Place the empty column in the microcentrifuge and centrifuge for an additional2 minutes at 9,000 x g (12,000 rpm) to dry the Ezup 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 Ezup spin column, since residual ethanol may interfere with subsequent reactions.This centrifugaatiostep ensures that no residual ethanol will be carried over during the following elution.

9. Add 50-100 µl Buffer CE directly onto the center part of EZ-10 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 elutiefficiency.

   NOTE 2: Elutionwith more than 100 µl (e.g. 200 µl) increases the DNA yield, but the concentrationwill be lower.

   NOTE 3: For maximum DNA yield, repeat elutiononce as described in this step.

   NOTE 4: A new microcentrifuge tube can be used for the second elutionstep to prevent dilutionof the first eluate.

   NOTE 5: For maximum DNA concentration, use the elu te in the microcentrifuge tube for the second elutionstep.

EZ-10 Column Plant Genomic DNA Purification Kit

Kit Contents

Component	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 ube	100
Protocol	1

Storage Conditions

EZ-10 columns and all buffers should be stored dry, at room temperature (15-25°C). The kit is stable for 1 yeas 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 afer 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 selectėly bound to the spin column and other impuritiessuch as proteins and salts do not bind to the column and are eliminated in flow through. No phenol extrractioand ethanol precipitatatioare required. The kit is also suitable for isolationof bacterial genomic DNA from colonies on dish. Purified genomic DNA is 20-50 kb in length.Purified DNA is suitable for downstream application such as RestrictionEndonuclease DigesstionsPCR, and other applicacations.

Features

- Fast and easy. Processing uses a rapid spin-column format. The entie 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
- Pipete tips
- Vortexer
- Isopropanol
- β-mercaptoethanol
- Ethanol (96-100%)
- RNase A (20 mg/ml, Optionalfor RNA-free DNA)
- Microcentrifuge tubes (1.5 ml or 2 ml)
- Water bath for heatingat 65°C

Before Starting

This protocol is designed for purificaatioof total DNA from plant. All centrifugagatisteps 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 PurificaatioKit 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 precipitatati before each use. If necessary, re-dissolve the precipitate by warming the solutionat 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 Solutionand Wash Solutio are supplied as concentrates. Before using for the first st time, add 24 ml isopropanol to 36 ml PW Solution,and 45ml ethanol to 15ml Wash Solution, respecti ely. Preheat the water bath or rocking plaaorm to 65°C.

Procedure

1. Grind 100 mg fresh plant tissue(or 20 mg dry plant tissueto 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 minutes.

   NOTE: If RNA-free genomic DNA is required, add 20 µl RNase A (20 mg/ml), mix by vortexing, and incubate for 2 minutes at room temperature before continuingwith step 3.
3. Add 0.6 ml of chloroform to the tube, mix well by inverting10 timesCentrifuge 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. Incubate at 70°C for 10 minutes.

5. Add 200 µl ethanol (96-100%), mix thoroughly by vortexing.
   NOTE: If a gelatinousmaterial 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 collectiontube. Centrifuge at 9,000 x g (12,000 rpm) for 1 minute. Discard the flow-through.
7. Add 500 µl PW Solution,and centrifuge for 1 minute at 9,000 x g (12,000 rpm).
   NOTE: Check the label to ensure PW Solutionwas diluted with isopropanol.
8. Add 500 µl Wash Solution,and centrifuge for 1 minute at 9,000 x g (12,000 rpm).
   NOTE: Discard the flow-through.
9. Place the empty column in the microcentrifuge and centrifuge for an additional2 minutes at 9,000 x g (12,000 rpm) to dry the EZ-10 membrane. Discard flowthrough 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 centrifugaatiostep 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 minute, and then centrifuge for 1 minute at 9,000 x g (12,000 rpm) to elute the DNA.
    NOTE 1: Warming the TE Buffer to 60°C will increase the elutiefficiency.
    NOTE2: For maximum DNA yield, repeat elutiononce as described in this step.
    NOTE 3: A new microcentrifuge tube can be used for the second elutionstep to prevent dilutionof the first eluate.
    NOTE 4: To maximize DNA concentration,use the eluate in the microcentrifuge tube for the second elutionstep.

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 Bu er(d)	5 ml	25 ml
EZ-10 Column (with 2.0-ml Collection ube)	50	250
Protocol	1	1

Notes

a. ACL Solutionmay form a precipitate upon storage. If necessary, dissolve the precipitate by warming the solutionto 37°C.

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 solutionat -20°C.

c. Before use, add 48 ml of 100% ethanol to 12 ml Wash Solutionfor BS427 or 120 ml of 100% ethanol to 30 ml Wash Solutionfor 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. ElutionBuffer 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 Animal Tissue

1. Cut up to 30 mg of tissueand place in a 1.5 ml centrifuge tube.
2. Add 300 µl of ACL Solution(Animal Cell Lysis Solutionto 1.5 ml centrifuge tube and 20 µl of Proteinase K.
3. Incubate at 55°C untl ssues are completely lysed (usually 1-3 hours). Vortex occasionally. Incubatingthe 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 minutes at room temperature before continuingwith step 5.
4. Cool to room temperature. Vortex for 20 seconds and centrifuge 10,000 x g (12,000 rpm) for 5 minutes.
5. Pipete 300 µl of supernatant to a new Eppendorf tube, add 300 µl of AB SolutionMix by occasionally invertingtube, and keep for 2 minutes. Then load all the solutionto an EZ-10 Spin Column.
6. Centrifuge at 2,000 x g (4,000 rpm) for 2 minutes and discard the flowthrough.
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 additionaminute to remove residual amount of Wash Solution.
10. Place the column into a clean 1.5 ml Eppendorf tube. Add 30-50 µl ElutionBuffer into the center part of membrane in the column. Incubate at RT for 2 or 3 minutes. Incubatingthe tube at 37°C or 50°C 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 °C.
13. Measure DNA quantity by UV absorption t A260 (1.0 OD unit is equivalent of 50 ug). Assess genomic DNA quality by an analytial 0.7% agarose gel.

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 Solutionto 1.5 ml centrifuge tubes and then add 20 µl of Proteinase K.
4. Incubate at 55°C 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 minutes at room temperature before continuingwith step 5.
5. Cool to room temperature. Vortex for 20 seconds and centrifuge at 10,000 x g (12,000 rpm) for 5 minutes.
6. Pipete 300 µl of supernatant into to a new Eppendorf tube, add 300 µl of AB Solution. Mix by occasionally invertingtube, and keep for 2 minutes. Then load all the solutioto 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 8.
10. Discard flow-through. Spin at 8,000 x g (10,000 rpm) for an additionaminute to remove residual amount of Wash Solution.
11. Place the column into a clean 1.5 ml Eppendorf tube. Add 30-50 µl Elution Bu er into the center part of membrane in the column. Incubate at RT for 2 or 3 minutes. Incubatingthe tube at 37°C or 50°C 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 -20°C.
14. Measure DNA quantityby UV absorptio at A260 (1.0 OD unit is equivalent of 50 µg). Assess genomic DNA quality by an analytial 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 timeswith PBS Solution.
4. Resuspend pellet in 300 µl of ACL solutionbuffer.
5. Add 20 µl of Proteinase K, mix well and Incubate at 55°C 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 minutes at room temperature before continuingwith step 6.
6. Cool to room temperature. Vortex for 20 seconds and centrifuge 10,000 x g (12,000 rpm) for 5 minutes.

7. Pipete 200 µl of supernatant to a new Eppendorf tube, add 200 µl of AB SolutionMix by occasionally invertingtube, and keep for 2 minutes. Then load all the solutio to a EZ-10 Spin Column.
8. Centrifuge at 2,000 x g (4,000 rpm) for 2 minutes and discard the flowthrough.
9. Add 500 µl of Wash Solution,and spin at 8,000 x g (10,000 rpm) for 1 minute.
10. Repeat Step 9.
11. Discard flow-through. Spin at 8,000 x g (10,000 rpm) for an additionaminute to remove residual amount of Wash Solution.
12. Place the column into a clean 1.5 ml Eppendorf tube. Add 30-50 µl ElutionBuffer into the center part of membrane in the column. Incubate at RT for 2 or 3 minutes. Incubatingthe tube at 37°C or 50°C 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°C.
15. Measure DNA quantity y UV absorpptiont A260 (1.0 OD unit is equivalent of 50 ug). Assess genomic DNA quality by an analytial 0.7% agarose gel.

From Paraffin Tissue

1. Excise 25~30 mg paraffin n tiss 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°C 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 Soluton (Animal Cell Lysis Soluuon) 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 minutes at room temperature before continuingwith step 11.
11. Incubate at 55 °C untl the ssue is completely lysed (usually 1-3 hours). Vortex occasionally. Incubaton in shaking water bath can reduce lysis me.
12. Cool to room temperature. Vortex for 20 seconds and centrifuge at 10,000 x g (12,000 rpm) for 5 minutes.

13. Pipette300 µl of supernatant to a new Eppendorf tube, add 300 µl of AB Soluuon. Mix by occasionally invertng tube, and keep for 2 minutes. Then load all the soluuon to a EZ-10 Spin Column.
14. Centrifuge at 2,000 x g (4,000 rpm) for 2 minutes and discard the flowthrough.
15. Add 500 µl of Wash Soluton, 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 addiional minute to remove residual amount of Wash Soluton.
18. Place the column into a clean 1.5 ml Eppendorf tube. Add 30-50 µl Eluton Buffer into the center part of membrane in the column. Incubate at RT for 2 or 3 minutes. Incubatng the tube at 37°C or 50°C 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°C.
21. Measure DNA quantty by UV absorppon at A260 (1.0 OD unit is equivalent of 50 µg). Assess genomic DNA quality by an analytcal 0.7% agarose gel.

EZ-10 Spin Column Genomic DNA Minipreps Kit, Blood

Kit Contents

Component	SK8253, 50 Preps	SK8254, 100 Preps
PBS Solution	8 ml	16 ml
Buffer CL (a)	12 ml	24 ml
Cw1 Solution(Conce trate) (b)	13 ml	26 ml
Cw2 Solution(Conce trate) (b)	9 ml	18 ml
CE Buffer (pH 9.0)	15 ml	30 ml
Bufffer TBP (optional)	50 ml	100 ml
Proteinase K(c)	1.2 ml	2.4 ml
EZ-10 Column (with 2.0-ml Collection ube)	50	100
Protocol	1	1

Notes

a. Buffer CL Solutio may form a precipitate upon storage. If necessary, dissolve the precipitate by warming the solutionto 56°C.

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 -20°C.

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 anti oagulant, and store tubes at 2-8°C. It is recommended to store blood samples less than 3 days as DNA degradationmay occur. For long term storage, collect blood in tubes containing a standard anti oagulant (preferably EDTA if high molecular weight DNA is required) and store at -80°C.

Blood Collection and Treatment

For every 1 ml of whole blood sample, add 0.1 ml of anti oagulant (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 Preparaton

A. Blood Samples (non-nucleated Erythrocytes, for example Human Blood): Collect ~100ul of blood into 2.0 ml centrifuge tube. Add PBS solutionto the tube to a final volume of 200ul. Vortex gently and let the tube stand for 1 minute 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 untilred cells lyse completely. Spin at 4,000 Xg (8,000 rpm) for 1 minute. Discard the supernatant carefully. Wash the precipitate with 500ul TE Buffer 2 times.Spin at 4,000 X g (8,000 rpm) for 1 minute 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 solutionto the tube to a final volume of 200ul. Vortex gently and let the tube stand for 1 minute 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 solutionand proceed to step 2.

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

- The solutionshould appear clear aaer complete lysis. If f soluti sn stappears cloudy, please extend incubation timununtlysis is complete and nd solutis 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 minutes at room temperature before continuingwith step 3.
- If final rreactio 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 afer additio 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 Collecton Tube. Let it stand at Room Temperature for 1-2minutes. Spin at 8,000 x g (10,000 rpm) for 2 minutes. Discard the flowthrough in the colleccon tube.
5. Add 500 µl of CW1 Soluton, 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 Soluton, 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 addiional minute to remove any residual amount of CW2 Soluton.
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. Incubatng the tube at 37°C or 50°C 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.
   - Small cloudy insoluble material may appear afer additio of ethanol, but this does not affect the performance of the kit. Proceed with step 4.
10. For long term storage, keep aliquots of purified genomic DNA at -20°C. Measure DNA quantty by UV absorppon at A260 (1.0 OD unit is equivalent of 50 µg). Assess genomic DNA quality by an analytcal 0.7% agarose gel.

Troubleshooting Guide

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 activityis weakened or lost. Add 30% additionaRNase A, and store e solutät 4°C.

Sample floats upon loading in agarose gel

The sample contains ethanol from washing step. Discard the liquid waste from the collectiontube aaer washing step, and spin again for r additiontwo minutes. Before re elution step, incubate the column at 50°C for ~5 minutes and allow ethanol to evaporate completely.