ONE-4-ALL Genomic DNA Mini-preps Kit Handbook

BS88503

BIO BASIC
Quality-Affordable Research

Introduction

ONE-4-ALL Genomic DNA Mini-Preps Kit is a designed for rapid purification of genomic DNA (as well as other forms of DNA, e.g. mitochondria) from fresh, frozen or paraffin-embedded animal tissues, cells, blood or bacteria.

Samples are first lysed using proteinase K in optimized buffer. The lysate is loaded onto the EZ-10 spin column, and DNA is selectively bound to EX membrane embedded in the column. During wash steps, protein and other impurities are removed and DNA is then eluted in 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/chloroform extraction or alcohol precipitation, and involves minimal handling. The whole procedures takes only 20 minutes after sample preparation.

Yields of DNA varies between samples depending on the amount and type of tissue, for most tissues 5-10 µg DNA can be obtain from 25 mg materials.

Features

- High quality of DNA, $OD_{260/OD280}$ of purified DNA is generally 1.7~1.9.
- Purification of high quality genomic DNA with a molecular weight≥20 kb.
- Fast and effective. Fast and easy processing using a rapid spin-column format.
- Compatible with many downstream applications such as PCR, restriction digestion and hybridization.
- No phenol/chloroform extraction or ethanol precipitation is required.
- High yield and reproductivity.
- The kit is fit for a variety of sample sources.

Kit Contents

Component	BS88503, 50 Preps	BS88504, 100 Preps	BS88505, 250 Preps
Buffer ACL	10 ml	20 ml	50 ml
Buffer CL	12 ml	24 ml	60 ml
CW1 Solution (concentrate)	13 ml	26 ml	65 ml
CW2 Solution (concentrate)	9 ml	18 ml	45 ml
CE Buffer (pH9.0)	15 ml	30 ml	2 x 30 ml
Proteinase K	1.25 ml	2 x 1.25 ml	5 x 1.25 ml
EZ-10 Spin Column (with 2.0 ml Collection Tube)	50	100	250
Protocol	1	1	1

NOTE 1: Buffer ACL and Buffer CL may form precipitate upon storage, warm to 56°C to dissolve it and cool down to room temperature before use.

NOTE 2: CW1 solution and CW2 solution are supplied as concentrates. Before use, add ethanol (96-100%) according to notes on page 4 to obtain a working solution.

Storage Conditions

EZ-10 spin 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 solution is stable at room temperature for 6 months, store at 2-8°C for long term storage.

Applications

Sample	Amount	Yield (µg)
Animal Tissues	25 mg	5-15
Cells	1-3 x 10^7	15-25
Blood	50-100 µl	1-3
Bacteria	1 ml	7-15

Quality Control

Each lot of ONE-4-ALL Genomic DNA Mini-Preps Kit is tested against predetermined specifications to ensure consistent product quality.

Materials Supplied by User

- Microcentrifuge capable of at least 12,000 × g
- Pipettes and pipette tips
- Vortexer
- Ethanol (96-100%)
- RNase A (10 mg/ml, Optional for RNA-free DNA)
- Microcentrifuge tubes (1.5 ml or 2 ml)
- Water bath for heating at 56°C

Before Starting

This protocol is designed for purification of total DNA from fresh, frozen or paraffin-embedded animal tissues, cells, blood, or bacteria. All centrifugation steps are carried out at room temperature (15-25°C) in a microcentrifuge. It is strongly recommended that you read this booklet throughly before starting. ONE-4-ALL Genomic DNA Mini-preps 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 Buffer ACL and 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.

Cw1 Solution and CW2 Solution are supplied as concentrates. Before using for the first time, add **17ml/35ml/85 ml** ethanol to **13ml/26ml/65ml** CW1 Solution; **21ml/42ml/105ml** ethanol to **9ml/18ml/45ml** CW2 Solution, respectively.

CW1 Solution

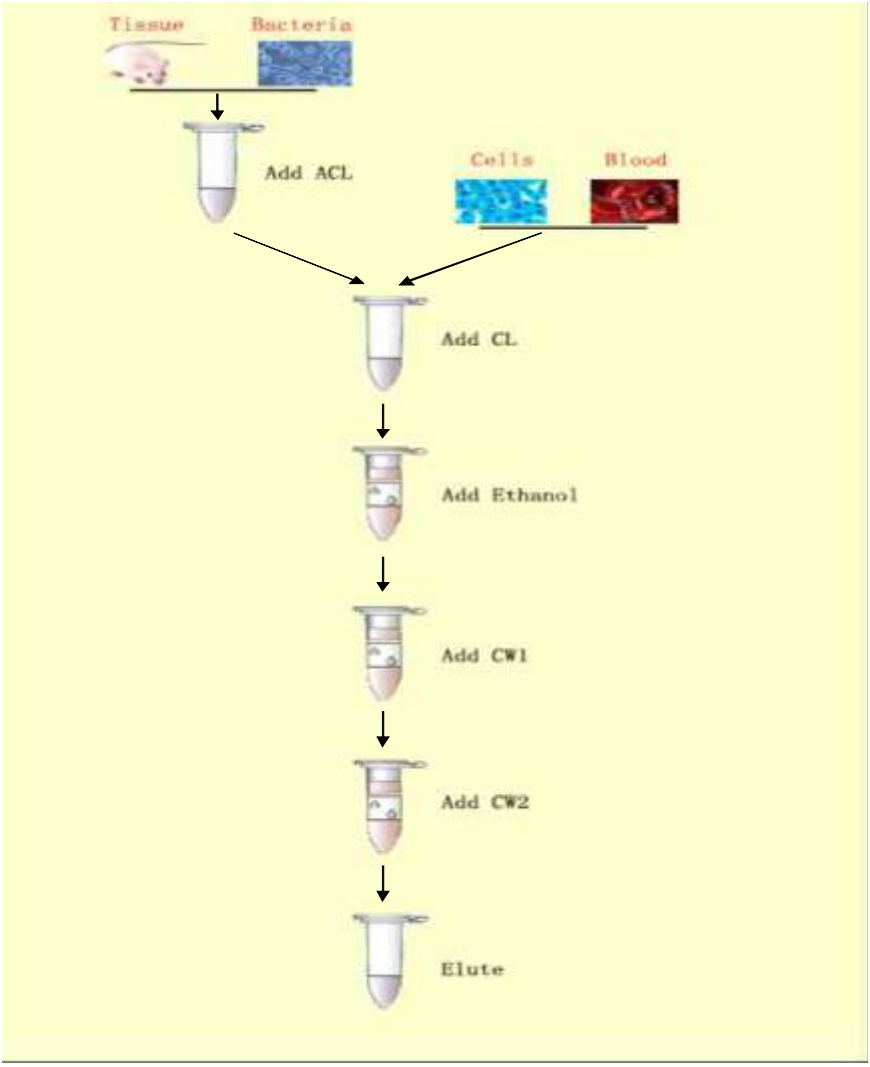
	BS88503, 50 Preps	BS88504, 100 Preps	BS88505, 250 Preps
CW1 Solution	13 ml	26 ml	65 ml
Ethanol (96-100%)	17 ml	34 ml	85 ml
CW1 Final Volume	30 ml	60 ml	150 ml

CW2 Solution

	BS88503, 50 Preps	BS88504, 100 Preps	BS88505, 250 Preps
CW2 Solution	9 ml	18 ml	65 ml
Ethanol (96-100%)	21ml	42 ml	85 ml
CW2 Final Volume	30 ml	60 ml	150 ml

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

Procedure


Tissue
Bacteria
Add ACL
Cells
Blood
Add CL
Add Ethanol
Add CW1
Add CW2
Elute

Notes for Sample Preparation

Sample collection and storage

Best results are obtained with fresh samples. If samples need to be stored before using, they should be frozen immediately and store at -20ºC for short term storage, or at -80ºC for long term storage. Avoid repeated freezing and thawing of stored samples, since this leads to degradation of DNA.

Collect blood in anticoagulant such as EDTA or ACD to keep the blood from clotting. However, heparin is not recommended, since it may bind to DNA during the purfication and inhibit amplification by PCR.

After proteinase K digestion, tissue samples can also be stored in Buffer ACL for up 6 months at ambient temperature without any reduction in DNA quality.

Starting amounts of samples

The yield and quality of genomic DNA depends on the amount of starting material. It is important not to exceed the capacity of lysis buffer and membrane. Use advisable amount of starting material as follow:

Sample	Amount
Muscle tissue	30 mg
Liver or brain tissue	20 mg
Kidney or spleen tissue	10 mg
Mammalian blood	100 µl
Bird or fish blood (with nucleated erythrocytes)	10 µl
Mouse tail	1.2 cm
Rat tail	0.6 cm
Cultured cells	5 x 10^6
Bacteria	2 x 10^9

Elution of Pure Nucleic Acids

Purified DNA is eluted from the EZ-10 spin column in CE Buffer.

Elution volume

For maximum DNA yield, collect the advisable volume of Buffer CE to elute.

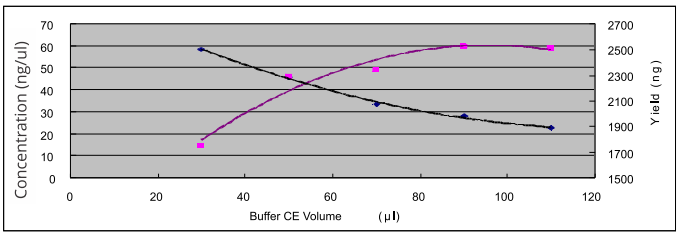


Figure 1. Relationship of concentration and yield in different elution volume.

Elution temperature

For maximum DNA yield, pre-warm Buffer CE to 60°C.

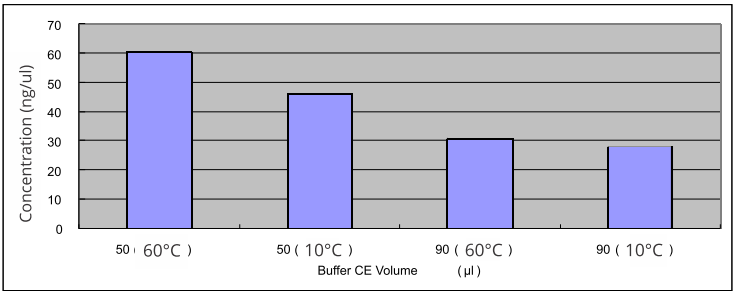


Figure 2. Impact of elution volume and temperature on DNA.

Elution steps

For maximum DNA yield, repeat elution once as described in this step. A new microcentrifuge tube can be used for the second elution step to prevent dilution of the first eluate. For maximum DNA concentration, use the eluate in the microcentrifuge tube for the second elution step.

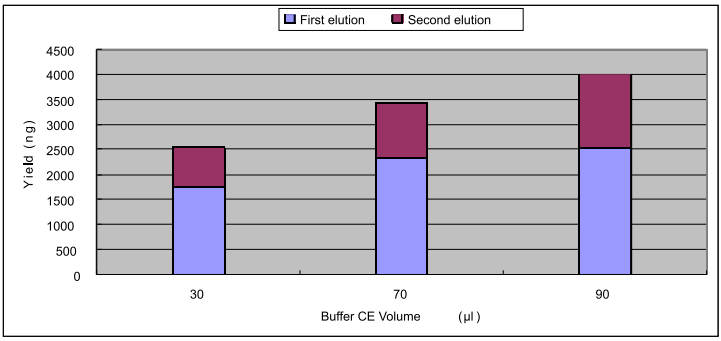


Figure 3. Elution efficiency of different elution buffer volume and steps.

Protocol (for purification of total DNA from fresh or frozen animal tissues)

Things to do before starting

- Buffer ACL and Buffer CL may form precipitates upon storage. If necessary, warm to 56°C to dissolve it before use.
- CW1 Solution and CW2 Solution are supplied as concentrates. Check if ethanol are added correctly.
- Preheat the water bath or rocking platform to 56°C.

Protocol

1. Cut up to 25 mg tissue into small pieces, grind in liquid nitrogen, transfer the powder to a 1.5 ml microcentrifuge tube and add 180 µl Buffer ACL, mix well.
   - Don't use more than 30 mg tissue, exceed starting materials will lead to lower yield.
   - For frozen tissue, avoid repeated thawing and freezing of samples since this will lead to DNA degradation.
   - For rodent tails, take 0.4~0.6 cm lengths of tail for rat and 1.0~1.2 cm for mouse.
   - For tissues with a very high number of cells, such as spleen, no more than 10 mg starting material should be used.
2. Add 20 µl proteinase K. Mix thoroughly by vortexing, and incubate at 56°C until the tissue is completely lysed.
   - Vortex occasionally during incubation to disperse the sample.
   - Lysis time varies depending on the type of tissue, typically 1~3 hours, or can be lysed overnight.
   - After incubation the lysate may appear viscous, but should not be gelatinous as it may clog the EZ-10 column. If the lysate appears very gelatinous, longer incubation is required.
   - If RNA-free genomic DNA is required, add 20 µl RNase A (10 mg/ml), mix by vortexing, and incubate for 5 minutes at room temperature before continuing with step 3.
3. Vortex for 15 seconds. Add 200 µl Buffer CL to the sample, and mix thoroughly by vortexing.
4. Add 200 µl ethanol (96-100%), and mix again thoroughly.
5. Transfer the mixture from step 4 (including any precipitate) into the EZ-10 Spin Column placed in a 2 ml collection tube. Centrifuge at 9,000 x g (12,000 rpm) for 1 minute. Discard the flow-through.
6. Add 500 µl CW1 Solution, and centrifuge for 1 minute at 9,000 x g (12,000 rpm). Discard the flow-through.
   - Check the label to ensure CW1 Solution was diluted with ethanol.
7. Add 500 µl CW2 Solution, and centrifuge for 1 minute at 9,000 x g (12,000 rpm). Discard the flow-through.
   - Check the label to ensure CW2 Solution was diluted with ethanol.
8. Place the empty column in the collection tube and centrifuge for an additional 2 minutes at 9,000 x g (12,000 rpm) to dry the membrane. Discard flow-through and transfer the spin column to a clean 1.5 ml centrifuge tube. Incubate the open spin column at room temperature for 2-3 min until the ethanol has completely evaporated.
   - 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.

9. Add 50-100 µl Buffer CE directly onto the center part of EZ-10 membrane. Incubate at room temperature for 2 minutes, and then centrifuge for 2 minutes at 9,000 x g (12,000 rpm) to elute the DNA.
   - Warm the Buffer CE to 60°C will increase the elution efficiency.
   - Elution with more than 100 µl (e.g. 200 µl) increases the DNA yield, but the concentration will be lower.
   - For maximum DNA yield, repeat elution once as described in this step.
   - A new microcentrifuge tube can be used for the second elution step to prevent dilution of the first eluate.
   - For maximum DNA concentration, use the eluate in the microcentrifuge tube for the second elution step.

Protocol (for purification of total DNA from cultured cells)

Materials Supplied by User (for cells)

- Prepare PBS Solution: 150 mM Sodium Chloride, 50 mM Potassium Phosphate, pH=7.2.
- Buffer ACL in the kit is not required for cell genomic DNA isolation.

Things to Do Before Starting

- Buffer CL may form precipitates upon storage. if necessary, warm to 56°C to dissolve it before use.
- CW1 Solution and CW2 Solution are supplied as concentrates. Check if ethanol are added correctly.
- Preheat the water bath or rocking platform to 56°C.

Procedure

1. Sample preparation (Cell Cultures)

   1a. Cells grown in suspension: Spin appropriate number of cells (max. 5 x 10^6) at 300 x g for 5 minutes at room temperature. Remove supernatant carefully. Add 200 µl PBS Solution, 200 µl Buffer CL and 20 µl proteinase K. Mix thoroughly by vortexing.

   1b. Cells grown in monolayer: Aspirate the medium and 200 µl Buffer PBS Solution to the cell-culture dish. Collect the lysate with a rubber policeman. Pipet the lysate into a microcentrifuge tube. Add 200 µl Buffer CL and 20 µl proteinase K. Mix thoroughly by vortexing.
   - If sample can not be used immediately for genomic DNA extraction, it is recommended to store at -80°C for long-term storage.
   - Avoid repeated freezing and thawing of stored samples, since this leads to degradation of DNA.

2. Incubate at 56°C for 10 minutes until the cells are completely lysed.
   - Vortex occasionally during incubation to disperse the sample.
   - If RNA-free genomic DNA is required, add 20 µl RNase A (10 mg/ml), mix by vortexing, and incubate for 5 minutes at room temperature before continuing with step 3.

3. Continue with step 4-9, page 8-9.

Protocol (for purification of total DNA from fresh or frozen anti-coagulated blood)

Materials Supplied by User (for blood)

- Prepare PBS Solution: 150 mM Sodium Chloride, 50 mM Potassium Phosphate, pH=7.2.
- Buffer ACL in the kit is not required for cell genomic DNA isolation.

Things to Do Before Starting

- Buffer CL may form precipitates upon storage. if necessary, warm to 56°C to dissolve it before use.
- CW1 Solution and CW2 Solution are supplied as concentrates. Check if ethanol are added correctly.
- Preheat the water bath or rocking platform to 56°C.

Procedure

1. Sample preparation (Blood)

   1a. Nonnucleated: Pipette 20 µl proteinase K into a 1.5 ml or 2 ml microcentrifuge tube. Add 50-100 µl anticoagulated blood. Adjust the volume to 220 µl with PBS. Continue with step 2.

   1b. Nucleated: Pipette 20 µl proteinase K into a 1.5 ml or 2 ml microcentrifuge tube. Add 5-10 µl anticoagulated blood. Adjust the volume to 220 µl with PBS. Continue with step 2.

   - If sample can not be used immediately for genomic DNA extraction, it is recommended to store at -80°C for long-term.
   - Avoid repeated freezing and thawing of stored samples, since this leads to degradation of DNA.

2. Add 200 µl Buffer CL to the sample, and mix thoroughly by vortexing. Incubate at 56°C for 10 minutes.
   - Vortex occasionally during incubation to disperse the sample.
   - If RNA-free genomic DNA is required, add 20 µl RNase A (10 mg/ml), mix by vortexing, and incubate for 5 minutes at room temperature before continuing with step 3.
3. Continue with step 4-9, page 8-9.

Protocol (for Embedded or Fixed samples)

Materials Supplied by User (for paraffin-embedded or formalin-fixed tissues)

- Xylene (for paraffin-embedded animal tissues)
- Prepare PBS Solution: 150 mM Sodium Chloride, 50 mM Potassium Phosphate, pH=7.2. (for formalin-fixed animal tissues)
- The length of DNA purified from fixed tissues is usually < 650 bp, depending on the type and age of the sample and the quality of the fixative used.

- Use of fixatives such as alcohol and formalin is recommended. Fixatives that cause cross-linking, such as osmic acid, are not recommended as it can be difficult to obtain amplifiable DNA from tissue fixed with these agents.
- Lysis time will vary from sample to sample depending on the type of tissue processed.
- Yields will depend both on the size and the age of the sample processed. Reduced yields compared with fresh or frozen tissues are to be expected.

Things to Do Before Starting

- Buffer ACL and Buffer CL may form precipitates upon storage. if necessary, warm to 56°C to dissolve it before use.
- CW1 Solution and CW2 Solution are supplied as concentrates. Check if ethanol is added correctly.
- Preheat the water bath or rocking platform to 56°C.

Procedure (for paraffin-embedded samples)

1. Place a small section (not more than 25 mg) of paraffin-embedded tissue in a 2 ml microcentrifuge tube (not provided).
2. Add 1200 µl xylene. Vortex vigorously.
3. Centrifuge in a microcentrifuge at full speed for 5 minutes at room temperature (15-25°C).
4. Remove supernatant by pipetting. Do not remove any of the pellet.
5. Add 1200 µl ethanol (96-100%) to the pellet to remove residual xylene, and mix gently by vortexing.
6. Centrifuge in a microcentrifuge at full speed for 5 minutes at room temperature.
7. Carefully remove the ethanol by pipetting. Do not remove any of the pellet.
8. Repeat steps 5-7 once.
9. Incubate the open microcentrifuge tube at 37°C for 10-15 minutes until the ethanol has evaporated.
10. Grind the the tissue pellet with pestles in 180 µl Buffer ACL and 20 µl proteinase K. Mix thoroughly by vortexing, and incubate at 56°C for 1-3 hours until the tissue is completely lysed.
    - Vortex occasionally during incubation to disperse the sample.
    - If RNA-free genomic DNA is required, add 20 µl RNase A (10 mg/ml), mix by vortexing, and incubate for 5 minutes at room temperature before continuing with step 11.
11. Continue with step 3-9, page 8-9.

Procedure (for formalin-fixed samples)

1. Place a small section (not more than 25 mg) of formalin-fixed tissue in a 1.5 ml microcentrifuge tube.
2. Wash the tissue with PBS solution to remove formalin.
3. Repeat step 2 once.

4. Grind the tissue pellet with pestles in 180 µl Buffer ACL and 20 µl proteinase K. Mix thoroughly by vortexing, and incubate at 56°C for 1-3 hours until the tissue is completely lysed.
   - Vortex occasionally during incubation to disperse the sample.
   - If RNA-free genomic DNA is required, add 20 µl RNase A (10 mg/ml), mix by vortexing, and incubate for 5 minutes at room temperature before continuing with step 5.
5. Continue with step 3-9, page 8-9.

Protocol (for purification of total DNA from Gram-negative or Gram-positive bacteria)

Materials Supplied by User (for bacteria)

- For Gram Positive bacteria, cell wall should be removed by an enzyme (e.g. Lysozyme) before lysis, but the enzyme is NOT supplied in the kit.
- Enzymatic lysis buffer
- 20 mM Tris·Cl, pH 8.0
- 2 mM sodium EDTA
- 1.2% Triton X-100
- Immediately before use, add lysozyme to 20 mg/ml

Things to Do Before Starting

- Buffer ACL and Buffer CL may form precipitates upon storage. if necessary, warm to 56°C to dissolve it before use.
- CW1 Solution and CW2 Solution are supplied as concentrates. Check if ethanol is added correctly.
- Preheat the water bath or rocking platform to 56°C.

Procedure (for bacteria)

1. Sample preparation (Cell Cultures)

   A. Gram-negative bacterials:

   1a. Transfer overnight culture (about 2 x 10^9 cells) into centrifuge tube and centrifuge at 10000 x g for 30 seconds, discard supernatant.

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

   1c. Add 200µl Buffer CL, and mix thoroughly by vortexing.

   B. Gram-positive bacterials:

   1a. Transfer overnight culture (about 2 x 10^9 cells) into centrifuge tube and centrifuge at 10000 x g for 30 seconds, discard supernatant.

   1b. 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.
   - Vortex occasionally during incubation to disperse the sample.
   - If RNA-free genomic DNA is required, add 20 µl RNase A (10 mg/ml), mix by vortexing, and incubate for 5 minutes at room temperature before continuing with step 2.

   1c. Add 200µl Buffer CL and 20µl proteinase K. Mix thoroughly by vortexing, and incubate at 56°C for 30 minutes.

2. Continue with step 4-9, page 8-9.

Protocol (for purification of total DNA from sperm)

Materials Supplied by User (for sperm)

- Prepare Buffer X2:
  - 20 mM Tris·Cl, pH 8.0
  - 20 mM EDTA
  - 200 mM NaCl
  - 4% SDS
- Immediately before use, add: 80 mM DTT, 12.5 µl/ml Proteinase K.
- Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.
- Store 1M DTT stock solution in aliquots at –20°C.
- Buffer ACL in the kit is not required for sperm genomic DNA isolation.

Things to Do Before Starting

- Prepare Buffer X2, as described above.
- Buffer ACL and Buffer CL may form precipitates upon storage. if necessary, warm to 56°C to dissolve it before use
- CW1 Solution and CW2 Solution are supplied as concentrates. Check if ethanol is added correctly.
- Preheat the water bath or rocking platform to 56°C.

Procedure (for sperm)

1. Place 100 µl sperm in a microcentrifuge tube and add 100 µl Buffer X2. Incubate at 56°C until the sample is dissolved (at least 1 hour). Vortex occasionally during incubation to disperse the sample, or place in a thermomixer, shaking water bath, or on a rocking platform.
2. Add 200 µl Buffer CL to the sample, and mix thoroughly by vortexing. Then add 200 µl ethanol (96–100%), and mix again thoroughly by vortexing.
   - It is essential that the sample, Buffer CL, and ethanol are mixed immediately and thoroughly by vortexing or pipetting to yield a homogeneous solution. Buffer CL and ethanol can be premixed and added together in one step to save time when processing multiple samples.
   - A white precipitate may form on addition of Buffer CL and ethanol. This precipitate does not interfere with the procedure.
3. Transfer the mixture from step 2 (including any precipitate) into the EZ-10 Spin column placed in a 2 ml collection tube. Centrifuge at ≥6000 x g (8000 rpm) for 1 minute. Discard flow-through.
4. Continue with step 6-9, page 8-9.

Troubleshooting Guide

Clumping in binding steps.

A. Make sure samples are homogenized completely before binding the spin column.
B. Reduce the amount of starting material. Use advisable amount of starting material as directed.

C. Insure samples are lysised completely. Lysis time varies depending on the type of tissue, typically 1~3 hours, or can be lysed overnight.
D. Check Buffer ACL and Buffer CL, if form precipitates upon storage, warm to 56°C to dissolve it before use.

Low yield.

A. Homogenize tissue completely. Treat the tissue samples with liquid nitrogen homogenizer.
B. Use advisable amount of starting material. DNA yield is dependent on the type, size, age, and storage of starting material. Please prepare samples as directed.
C. Check the label to ensure CW1, CW2 Solution was diluted ethanol.
D. Elution step has to be strictly followed. Please reference the direction on page 6-7.
E. The membrane have been overdried. Incubate the membrane at room temperature 3-5 minutes to dry the membrane of the EZ-10 spin column. Do not leave the membrane at 65°C for long-term.

Blood forms clots, lysate does not pass through the column or low yield.

A. Reduce the amount of blood. Use advisable amount of starting material as directed.
B. Collect blood in anticoagulant such as EDTA or ACD to keep the blood from clotting.
C. The amount of DNA isolated from a blood sample is highly dependent on the number of white blood cells present in the sample and the blood storage conditions. Samples frozen at -80°C typically exhibit a 15% decrease in DNA yield. For best results, the blood should be stored at 4°C for less than 5 days or be frozen at -80°C on the same day.

Inhibition of downstream enzymatic reactions.

A. Residual ethanol from the CW2 Solution can inhibit downstream enzymatic reactions. Centrifuge the column at 12,000 × g for 2 minutes and incubate the open spin column at room temperature for 2-3 minutes to remove the residual ethanol throughly.
B. Residual salt can inhibit downstream enzymatic reactions. Ensure that Buffer CW1, CW2 have been used at room temperature (15–25°C).
C. Reduce the temple of genomic DNA for PCR applications, a single-copy gene can typically be detected after 35 PCR cycles with 100 ng template DNA .

DNA Degradation.

A. Use fresh sample. For frozen samples, ensure that they were flash-frozen immediately in liquid nitrogen and properly stored at -70°C.
B. Old samples often yield only degraded DNA.

A_{260}/A_{280} ratio of purified DNA is high.

A. DNA is contaminated with RNA, perform the optional RNase treatment in the protocol.

RNA-Contaminations of DNA.

A. Perform optional RNase treatment in the protocol.
B. Add RNase to the DNA elution directly.

DNA degradation after storage.

A. DNA can be stored at 2-4°C for several weeks. For long term storage, DNA should be stored at -20°C.
B. Elute DNA with CE buffer or TE buffer, Tris or Tris-EDTA buffer contains sufficient buffering capacity to prevent acid hydrolysis.