Ver 1.0

Handbook for

Clinic SV
(Mini/Midi/Maxi)

Exgene™

DNA PURIFICATION HANDBOOK

CE
IVD

GeneAll

Customer & Technical Support

Should you have any further questions, do not hesitate to contact us.

We appreciate your comments and advice.

Contact Information

www.geneall.com

Tel : 82-2-407-0096

Fax : 82-2-407-0779

Sales Email: sales@geneall.com

Technical Information Email: tech@geneall.com

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This protocol handbook is included in:

GeneAll® Exgene™ Clinic SV Mini (108-101, 108-152)
GeneAll® Exgene™ Clinic SV Midi (108-226, 108-201)
GeneAll® Exgene™ Clinic SV Maxi (108-310, 108-326)

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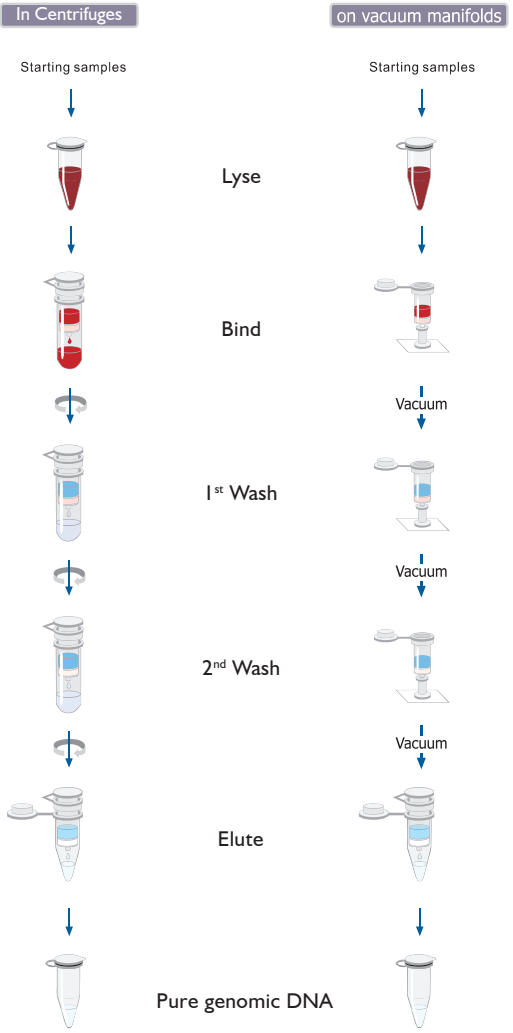
Brief protocol

Protocol for Blood / Cultured Cells



1. Add 20 μl of Proteinase K solution
2. Transfer 200 μl of the sample
3. (Optional) Add 20 μl of RNase A solution and incubate for 2 min at RT
4. Add 200 μl of Buffer BL
5. Incubate for 10 min at 56 °C
6. Add 200 μl of absolute ethanol

7. Transfer the mixture into mini column
8. Centrifuge for 1 min, ≥ 6000 xg

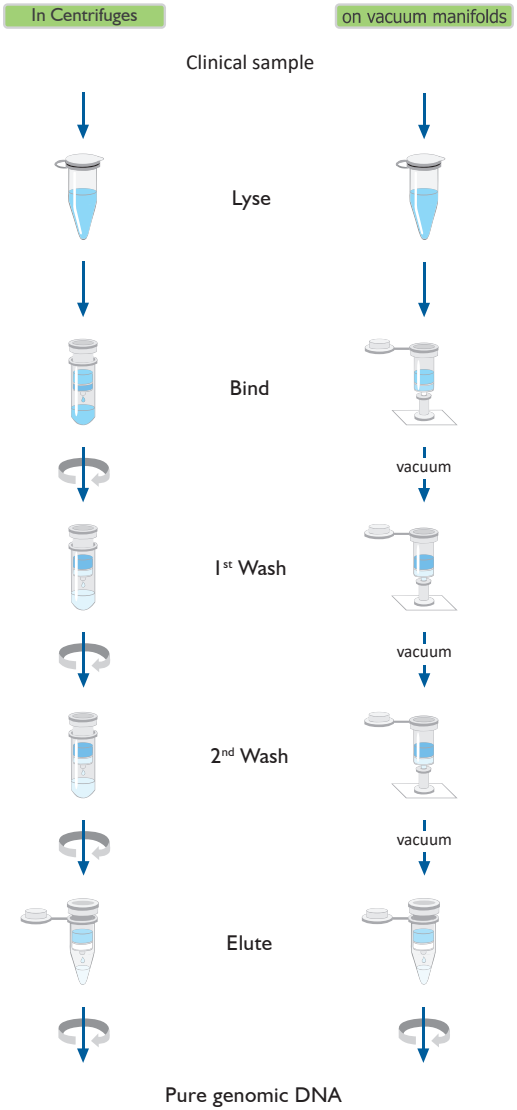
9. Add 600 μl of Buffer BW into mini column
10. Centrifuge for 1 min, ≥ 6000 xg

11. Add 700 μl of Buffer TW into mini column
12. centrifuge for 1 min, ≥ 6000 xg
13. Additional centrifuge for 1 min ≥ 13000 xg

14. Apply 200 μl Buffer AE into mini column
15. Incubate for 1 min at RT
16. Centrifuge for 1 min, ≥ 13000 xg

Brief protocol

Protocol for Tissue



1. Homogenize the tissue sample

2. Add 200 µl of Buffer CL
3. Add 20 µl of Proteinase K solution
4. Incubate at 56 °C until completely lysed
5. (Optional) Add 20 µl of RNase A solution and incubate for 2 min at RT
6. Add 200 µl of Buffer BL
7. Incubate for 10 min at 70 °C

8. Transfer the mixture to mini column
9. Centrifuge for 1 min, ≥ 6000 xg

10. Add 600 µl of Buffer BW into mini column
11. Centrifuge for 1 min, ≥ 6000 xg

12. Add 700 µl of Buffer TW into mini column
13. Centrifuge for 1 min, ≥ 6000 xg
14. Additional centrifuge for 1 min, ≥ 13000 xg

15. Apply 200 µl Buffer AE into mini column
16. Incubate for 1 min at RT
17. Centrifuge for 1 min, ≥ 13000 xg

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Protocols

Kit Contents

Components	Mini		Midi		Maxi		Storage
Cat. No.	108-101	108-152	108-226	108-201	108-310	108-326	
No. of preparation (test)	100	250	26	100	10	26	Room temperature (15 °C to 25 °C)
Buffer CL (ml)	25	60	60	200	60	200	
Buffer BL (ml)	25	60	80	150 x 2 ea	150	160 x 2 ea	
Buffer BW (ml) (concentrate) *	40	90	40	90 x 2 ea	40	90	
Buffer TW (ml) (concentrate) *	24	50	24	50 x 2 ea	24	36 x 2 ea	
Buffer AE (ml) **	30	60	30	120	60	60 x 2 ea	
Proteinase K (mg) ***	48	120	60	120 x 2 ea	48	120	
PK-Storage Buffer (ml)	4	7	4	7 x 2 ea	4	7	
Column Type G (with Collection Tube) (ea)	100	250	26	100	10	26	
Collection Tube (ea)	200	500	26	100	10	26	
IFU (ea)	1	1	1	1	1	1	

** Before the first use, add the appropriate amount of absolute ethanol (ACS grade quality or higher) to Buffer BW and TW as indicated on the bottle.*

*** 10 mM Tris-HCl, pH 9.0, 0.5 mM EDTA.*

**** After reconstitution of Proteinase K store at 4 °C. For the long-term storage of Proteinase K, store at -20 °C. For more detail, please refer to the instruction of Proteinase K on page 8.*

Materials to Be Supplied by the User

- Reagent: Absolute ethanol (ACS grade quality or higher)
- Disposable material: Sterile pipette tips, Disposable gloves, Sterile centrifuge tubes, Serological pipette
- Equipment: Swing bucket centrifuge, Vortex mixer, Heat block or Dry oven, Suitable protector, Pipet aid

Product Specifications

Exgene™ Clinic SV			
Type	Spin/Vacuum		
Size	Mini	Midi	Maxi
Preparation time	≥ 30 min	≥ 60 min	≥ 60 min
Maximum loading volume	750 µl	4 ml	20 ml
Minimum elution volume	30 µl	100 µl	300 µl

Quality Control

All components in Exgene™ Clinic SV series are manufactured and maintained in a state of strict cleanliness.

Rigorous quality control is performed consistently across batches, and only the kits meeting the required standards authorized for delivery.

Storage Conditions

All components of Exgene™ Clinic SV series should be stored at room temperature (15 °C to 25 °C) and protected from direct sunlight exposure.

During shipment or storage under cool ambient condition, a precipitate may formed in Buffer BL or BW. In such a case, Incubate bottle at 56 °C prior to use to dissolve precipitates.

Using precipitated buffers will lead to poor DNA recovery. Exgene™ Clinic SV mini is guaranteed until the expiration date printed on the product box.

Safety Information

The buffers included in Exgene™ Clinic SV series contain irritants that can be harmful upon contact with skin or eyes, inhalation or ingestion. Care should be taken when handling such materials. Always wear gloves and eye protection, and follow standard safety precautions.

Buffer CL, BL and BW contains chaotropic agents, which can form highly reactive compounds when combined with bleach.

DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

Product Disclaimer

Exgene™ Clinic SV series are intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

Preventing Contamination

Proper microbiological, aseptic technique should always be used when working with trace or evidentiary materials.

Always wear disposable gloves while handling reagents and samples. The use of sterile tip, tube and other instruments is recommended throughout the procedure.

Proteinase K

Exgene™ Clinic SV series contain Proteinase K to maximize recovery and yield from a various samples.

Add PK-Storage Buffer to one tube of lyophilized Proteinase K, and gently invert to dissolve.

Store Proteinase K Solution at 4 °C. For long term storage, we suggest storing it at -20 °C.

Description

The Exgene™ Clinic SV (Mini / Midi / Maxi) series are designed for the extraction of genomic DNA from various clinical samples. Utilizing advanced silica-binding technology, these Kits efficiently extract DNA for a wide range of applications.

The process begins by lysing samples in an optimized buffer containing detergent and a lytic enzyme. Under ideal binding conditions, DNA in the lysate binds to a silica membrane, while impurities pass through into a collection tube.

The membranes are then washed with a series of alcohol-containing buffers to remove any traces of proteins, cellular debris, and salts. Finally, the purified DNA is eluted into a clean tube with deionized water of low ionic strength buffer.

This purified DNA can be directly used for various downstream applications such as PCR, NGS, Sanger-sequencing.

Intended Purpose

Exgene™ Clinic SV (Mini / Midi / Maxi) series provide fast and easy methods of genomic DNA from clinical samples, such as blood, tissue, swab, body fluid, cultured cell, dried blood spot, bacteria, Saliva, Mouth wash, Hair, and etc.

Warning and Precautions

- Should be used for in vitro diagnostics.
- Intended for professional use.
- Read and follow the instructions manual before using the product.
- Be sure to wear personal protective equipment such as gloves and goggles when using this product.
- Do not dispose of Buffer CL, Buffer BL and Buffer BW with bleach or acidic substances, as they contain irritants.
- Buffer BW and Buffer TW contain large amounts of alcohol, so keep them away from fire.
- Store the product at the specified storage temperature and do not use it past its expiration date.
- Buffer CL and Buffer BL must be checked for precipitation before use, and if precipitation occurs, it must be completely dissolved at 56 °C before use.

* A notice to the user that any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

A PROTOCOL FOR

Whole blood / body fluid / cultured cells using microcentrifuge

Before experiment

- Before first use, add absolute ethanol (ACS grade or better) into Buffer BW and TW as indicated on the bottle.
- Prepare 1.5 ml microcentrifuge tube.
- Prepare heat block or water bath to 56 °C.
- Prepare absolute ethanol.
- All centrifugation should be performed at room temperature.
- Prepare Proteinase K solution (20 mg/ml) for first use.
- If a precipitate has formed in Buffer CL and BL, heat to dissolve at 56 °C before use.

1. **Add 20 μl of Proteinase K solution (20 mg/ml, provided) into the bottom of a 1.5 ml microcentrifuge tube (not provided).**
   If the sample volume is larger than 200 μl, increase the amount of Proteinase K proportionally. When the concentration of cells is low, up to 400 μl of starting sample can be used. For 400 μl of sample volume, 40 μl of Proteinase K solution is needed.

2. **Transfer 200 μl of sample to the tube. Use the starting sample listed below.**
   If the sample volume is less than 200 μl, adjust the volume to 200 μl with 1 X PBS.

Sample	Max. amount per prep	Preparation
Mammalian whole blood	200 μl	Direct use
Body fluid	200 μl	Direct use
Buffy coat	200 μl	Direct use
Nucleated blood of bird, fish, reptile and amphibian	10 μl	10 μl blood with 190 μl of 1 X PBS
Cultured cells or lymphocyte	5 x 10^6 cells	5 x 10^6 cells in 200 μl of 1 X PBS
Virus	200 μl	200 μl of virus-containing media

3. ***(Optional :)* If RNA-free DNA is required, add 20 μl or RNase A solution (20 mg/ml, Cat. No. 391-001, not provided) to the sample, pipet 2 to 3 times to mix and incubate at room temperature for 2 min.**
   Unless RNase A is treated, RNA will be co-purified with DNA. RNA can inhibit some downstream enzymatic reactions, but will not inhibit PCR itself.

4. **Add 200 μl of Buffer BL to the tube. Vortex the tube to mix thoroughly. Incubate at 56 ˚C for 10 min. After incubation, spin down the tube briefly to remove any drops from inside of the lid.**
   If the sample volume is larger than 200 μl, increase the volume of Buffer BL in proportion. Ratio of Buffer BL to the starting sample volume is 1:1.

5. **Add 200 μl of absolute ethanol (not provided) to the sample, pulse vortex to mix the sample thoroughly, and spin down briefly to remove any drops from inside of the lid.**
   If the sample volume is larger than 200 μl, increase the ethanol volume proportionally.

6. **Transfer the mixture to the Column Type G (mini) carefully, centrifuge at 6000 xg above (> 8000 rpm) for 1 min, and replace the collection tube with new one (provided).**
   If starting sample volume is larger than 200 μl, apply the mixture twice; apply 700 μl of the mixture, spin down, discard the pass-through, re-insert empty collection tube, and repeat this step again until all of the mixture has applied to the mini column.
   If the mixture has not passed completely through the membrane, centrifuge again at full speed (>13000 xg) until all of the solution has passed through. Centrifugation at full speed is recommended to avoid clogging especially when applying the sample with high-cell density, such as buffy coat, lymphocyte or cultured cells. Centrifugation at full speed will not affect DNA recovery.

7. **Add 600 μl of Buffer BW, centrifuge at 6000 xg above (> 8000 rpm) for 1 min and replace the collection tube with new one (provided).**
   If the mini column has colored residue after centrifuge, repeat this step until no colored residue remain. See Troubleshooting guide for detail.
   Centrifugation at full speed will not affect DNA recovery.

8. **Apply 700 μl of Buffer TW. Centrifuge at 6000 xg above (> 8000 rpm) for 1 min. Discard the pass-through and re-insert the mini column back into the collection tube.**
   Centrifugation at full speed will not affect DNA recovery.

9. **Centrifuge at full speed for 1 min to remove residual wash buffer. Place the mini column in a fresh 1.5 ml microcentrifuge tube (not provided).**
   Care must be taken at this step for eliminating the carryover of Buffer TW.
   If a carryover of Buffer TW still occurs, centrifuge again at full speed for 1 min with the collection tube before transferring to a new 1.5 ml microcentrifuge tube.
   Centrifugation must be performed at full speed (13000 xg to 20000 xg).

10. **Add 200 μl of Buffer AE or sterilized water. Incubate at room temperature for 1 min. After incubation, centrifuge at full speed for 1 min.**
    * For low cell-density sample, such as body fluids or virus, use 50 μl to 150 μl elution buffer as based on the species and conditions of starting sample or the downstream applications.
    For long-term storage, eluting in Buffer AE is recommended. But EDTA included in Buffer AE can inhibit subsequent enzymatic reactions, so you can avoid such latent problem by using distilled deionized water (> pH 7.0) or Tris-HCl (> pH 8.5). When using water for elution, check the pH of water before elution.

B PROTOCOL FOR

Buccal swab

Before experiment

- Before first use, add absolute ethanol (ACS grade or better) into Buffer BW and TW as indicated on the bottle.
- Prepare 1.5 ml microcentrifuge tube and 2.0 ml microcentrifuge tube.
- Prepare heat block or water bath to 56 °C.
- Prepare sterile sharp blade (or wire cutter) and tweezer.
- Prepare 1 X PBS and absolute ethanol.
- All centrifugation should be performed at room temperature.
- Prepare Proteinase K solution (20 mg/ml) for first use.
- If a precipitate has formed in Buffer CL and BL, heat to dissolve at 56 °C before use.

1. **Scrape the swab firmly more than 5 to 6 times against the inside of cheek.**
   To avoid contamination from other materials, ensure that the person who provides the sample has not taken any food or drink in 30 min prior to sample collection.

2. **Place the swab in 2.0 ml microcentrifuge tube (not provided). Clip off handle of brush with sterile sharp blade or wire cutter. Add 400 µl of 1 X PBS to the tube.**
   Cutters should be rinsed with 70 % ethanol to prevent contamination between samples.

3. ***(Optional :)* If RNA-free DNA is required, add 20 µl of RNase A solution (20 mg/ml, Cat. No. 391-001, not provided), vortex to mix, and incubate at room temperature for 2 min.**
   Unless RNase A is treated, RNA will be co-purified with DNA. RNA may inhibit some downstream enzymatic reactions, but will not inhibit PCR itself.

4. **Add 20 µl of Proteinase K solution (20 mg/ml, provided) and 400 µl of Buffer BL to the sample. Vortex vigorously to mix immediately. For efficient lysis, mix the sample completely.**

5. **Incubate at 56 ˚C for 10 min. After incubation, spin down the tube briefly to remove any drops from inside of the lid.**

6. **Add 400 μl of absolute ethanol (not provided) to the lysate, and mix well by vortexing.After incubation, briefly spin down the tube to remove any drops from inside the lid.**

7. **Transfer carefully up to 700 μl of the mixture to the Column Type G (mini). Close the cap. Centrifuge at 6000 xg above (> 8000 rpm) for 1 min. Discard the pass-through and re-insert the mini column back into the collection tube.**

8. **Repeat step 7 until all the remaining mixture has been applied to the mini column. Replace the collection tube with new one (provided).**

9. **Continue with step 7 in A. PROTOCOL FOR Whole blood / body fluid / cultured cell using microcentrifuge on page 11.**

C PROTOCOL FOR

Saliva / mouth wash

Before experiment

- Before first use, add absolute ethanol (ACS grade or better) into Buffer BW and TW as indicated on the bottle.
- Prepare 1.5 ml microcentrifuge tube and 50 ml conical tube.
- Prepare heat block or water bath to 56 °C.
- Prepare sterile sharp blade (or wire cutter) and tweezer.
- Prepare 1 X PBS and absolute ethanol.
- All centrifugation should be performed at room temperature.
- Prepare Proteinase K solution (20 mg/ml) for first use.
- If a precipitate has formed in Buffer CL and BL, heat to dissolve at 56 °C before use.

1. **Collect 10 ml of mouthwash in a 50 ml conical tube (not provided), or collect 1 ml of saliva by spitting in a 50 ml conical tube. If saliva is used, add 5 ml of 1 X PBS to the sample and vortex to mix.**
   To avoid contamination from other materials, ensure that the person who provides the sample has not taken any food or drink in the 30 min prior to sample collection.

2. **Centrifuge at 2000 x*g* (3000 rpm) for 5 min to pellet cells. Immediately and carefully decant the supernatant to prevent loose cell pellets. Re-suspend completely the pellets in 200 µl of 1 X PBS.**
   If the pellets are loose, repeat centrifugation.

3. ***(Optional :)* If RNA-free DNA is required, add 20 µl of RNase A solution (20 mg/ml, Cat. No. 391-001, not provided), vortex to mix, and incubate at room temperature for 2 min.**
   Unless RNase A is treated, RNA will be co-purified with DNA. RNA can inhibit some downstream enzymatic reactions, but will not inhibit PCR itself.

4. **Add 20 µl of Proteinase K solution (20 mg/ml, provided) and 200 µl of Buffer BL to the sample. Vortex vigorously to mix completely. Incubate at 56 °C for 10 min. After incubation, spin down the tube briefly to remove any drops from inside of the lid.**
   For efficient lysis, mix the sample completely.

5. **Continue with step 5 in A. PROTOCOL FOR Whole blood / body fluid / cultured cell using microcentrifuge on page 11.**

D PROTOCOL FOR
Hair

Before experiment

- Before first use, add absolute ethanol (ACS grade or better) into Buffer BW and TW as indicated on the bottle.
- Prepare heat block or water bath to 56 °C.
- Prepare 1.5 ml microcentrifuge tube.
- Prepare absolute ethanol.
- Prepare Buffer H as follow;
10 mM Tris-HCl, pH 8.0, 10 mM EDTA, 100 mM NaCl, 2 % SDS, 40 mM DTT
(Add DTT immediately before use, because it oxidizes quickly in aqueous solutions.)
- Equilibrate Buffer AE to room temperature.
- All centrifugation should be performed at room temperature.
- If a precipitate has formed in Buffer BL, heat to dissolve at 56 °C.

1. **Collect hair sample in a 1.5 ml microcentrifuge tube (not provided). The amount of starting sample should not exceed 30 mg.**
   It is recommended to use 0.5 cm to 1 cm from the root ends of plucked hair samples.

2. **Add 180 µl of prepared Buffer H and 20 µl of Proteinase K solution (20 mg/ml, provided) to the tube, and vortex to mix thoroughly.**

3. **Incubate at 56 °C for at least 1 hour until the sample is dissolved. After incubation, Spin down the tube briefly to remove any drops from inside of the lid.**
   Invert the tube occasionally to disperse the sample, or place on a rocking platform. Hair follicles should be completely dissolved, however hair shaft may be not dissolved completely and this residual solid materials will not affect DNA recovery.

4. **Continue with step 3 in A. PROTOCOL FOR Whole blood / body fluid / cultured cell using microcentrifuge on page 11.**

E PROTOCOL FOR
Sperm

Before experiment

- Before first use, add absolute ethanol (ACS grade or better) into Buffer BW and TW as indicated on the bottle.
- Prepare heat block or water bath to 56 °C.
- Prepare 1.5 ml microcentrifuge tube.
- Prepare absolute ethanol.
- Prepare Buffer H2 as follow;
20 mM Tris-HCl, pH 8.0, 20 mM EDTA, 200 mM NaCl, 4 % SDS, 80 mM DTT
(Add DTT immediately before use, because it oxidizes quickly in aqueous solutions.)
- Equilibrate Buffer AE to room temperature.
- All centrifugation should be performed at room temperature.
- If a precipitate has formed in Buffer BL, heat to dissolve at 56 °C.

1. **Place 100 µl of sperm in a 1.5 ml microcentrifuge tube (not provided). Add 100 µl of Buffer H2 and 20 µl of Proteinase K solution (20 mg/ml, provided) to the tube. Mix thoroughly by vortexing.**

2. **Incubate at 56 °C until the sample is dissolved completely. After incubation, spin down the tube briefly to remove any drops from inside of the lid.**
   It may need at least 1 hour for complete lysis.
   Invert the tube occasionally to disperse the sample, or place on a rocking platform.

3. **Continue with step 3 in A. PROTOCOL FOR Whole blood / body fluid / cultured cell using microcentrifuge on page 11.**

F PROTOCOL FOR

Whole blood / body fluid using vacuum

Before experiment

- Before first use, add absolute ethanol (ACS grade or better) into Buffer BW and TW as indicated on the bottle.
- Prepare heat block or water bath to 56 °C.
- Prepare absolute ethanol.
- Prepare 1.5 ml microcentrifuge tube.
- Prepare vacuum system; manifold, trap, tubing and pump capable of 15 to 20 inchHg
- Equilibrate Buffer AE to room temperature.
- All centrifugation should be performed at room temperature.
- If a precipitate has formed in Buffer BL, heat to dissolve at 56 °C before use.

1. **Add 20 μl of Proteinase K solution (20 mg/ml, provided) into the bottom of a 1.5 ml microcentrifuge tube (not provided).**
   If the sample volume is larger than 200 μl, increase the amount of Proteinase K proportionally.
   When the concentration of cells is low, up to 400 μl of starting sample can be used. For 400 μl of sample volume, 40 μl of Proteinase K solution is needed.

2. **Transfer 200 μl of sample to the tube. Use the starting sample listed below.**
   If the sample volume is less than 200 μl, adjust the volume to 200 μl with 1 X PBS.

Sample	Max. amount per prep	Preparation
Mammalian whole blood	200 μl	Direct use
Body fluid	200 μl	Direct use
Buffy coat	200 μl	Direct use
Nucleated blood of bird, fish, reptile and amphibian	10 μl	10 μl blood with 190 μl of 1 X PBS
Cultured cells or lymphocyte	5 x 10^6 cells	5 x 10^6 cells in 200 μl of 1 X PBS
Virus	200 μl	200 μl of virus-containing media

3. ***(Optional :)* If RNA-free DNA is required, add 20 μl of RNase A solution (20 mg/ml, Cat. No. 391-001, not provided) to the sample, pipet 2 to 3 times to mix and incubate at room temperature for 2 min.**
   Unless RNase A is treated, RNA will be copurified with DNA. RNA can inhibit some downstream enzymatic reactions, but will not inhibit PCR itself.

4. **Add 200 μl of Buffer BL to the tube. Vortex the tube to mix thoroughly. Incubate at 56 ˚C for 10 min. Spin down briefly to remove any drops from inside of the lid.**
   If the sample volume is larger than 200 μl, increase the volume of Buffer BL in proportion. Ratio of Buffer BL to the starting sample volume is 1:1.
   It is essential to mix the sample and Buffer BL thoroughly for good result.

5. **Add 200 μl of absolute ethanol (not provided) to the sample, pulse-vortex to mix the sample thoroughly, and spin down briefly to remove any drops from inside of the lid.**
   If the sample volume is larger than 200 μl, increase the ethanol volume proportionally.

6. **Attach the Column Type G (mini) to a port of the vacuum manifold tightly. If available, use vacuum adaptors to avoid cross-contamination between the samples.**
   Most commercial vacuum manifold with luer connectors can be adopted to this protocol.
   If the mini column becomes clogged during this procedure, it is possible to switch to the procedure for purification by centrifugation (page 11).

7. **Transfer the mixture to the mini column by pipetting. Switch on vacuum source to draw the solution through the mini column. When all liquid has been pulled through the mini column, release the vacuum.**
   If starting sample volume is larger than 200 μl, repeat this step until all of mixture has applied to the mini column.
   If the mixture has not passed completely through the membrane, you can switch to centrifugation protocol by step 6 at page 11.

8. **Apply 600 μl of Buffer BW and switch on vacuum source. When all liquid has been pulled through the mini column, release the vacuum.**
   If the mini column has colored residue after this step, repeat this step until no colored residue remain. See Troubleshooting guide for detail.

9. **Apply 700 μl of Buffer TW and switch on vacuum source. When all liquid has been pulled through the mini column, release the vacuum. Transfer the mini column into a empty collection tube (provided).**

10. **Continue with step 9 in A. PROTOCOL FOR Whole blood / body fluid / cultured cell using microcentrifuge on page 11.**

G PROTOCOL FOR
Animal tissue

Before experiment

- Before first use, add absolute ethanol (ACS grade or better) into Buffer BW and TW as indicated on the bottle.
- Prepare heat block or water bath to 56 °C and 70 °C.
- Prepare 1.5 ml microcentrifuge tube.
- Equilibrate Buffer AE to room temperature.
- All centrifugation should be performed at room temperature.
- Buffer BL and CL may precipitate at cool ambient temperature. If so, dissolve it in 56 °C water bath.

1. Homogenize up to 20 mg of tissue as described in step 1a, 1b or 1c, depending on the sample type.
Homogenizing the sample finely will accelerate lysis and decrease the lysis time.
For spleen tissue, up to 10 mg can be processed.

1a. For soft tissue, such as liver or brain, put up to 20 mg of the tissue into 1.5 ml microcentrifuge tube (not provided), add 200 µl of Buffer CL, and homogenize thoroughly with microhomogenizer.
Homogenize carefully for minimization of foaming.

1b. If micro homogenizer is not available or the tissue is not soft, grind the tissue to a fine powder with liquid nitrogen in a pre-chilled mortar and pestle. Put up to 20 mg of the powdered tissue into 1.5 ml microcentrifuge tube. Add 200 µl of Buffer CL and pulse-vortex for 15 s.

1c. If neither 1a nor 1b is available, mince the tissue with sharp blade or scalpel as small as possible. Put the tissue into a 1.5 ml microcentrifuge tube.
Add 200 µl of Buffer CL and pulse-vortex for 15 s.

** ***Alternatively, tissue samples can be effectively homogenized using some instruments, such as a rotor-stator homogenizer or a bead-beater.***

2. **Add 20 μl of Proteinase K solution (20 mg/ml, provided). Mix completely by vortexing or pipetting. Incubate at 56 ˚C until the sample is completely lysed. Spin down the tube briefly to remove any drops from inside of the lid.**

   It is essential to mix the components completely for proper lysis.

   Lysis time varies from 10 min to 3 hours usually depending on the type of tissue and the homogenization method (step 1). The lysate should become translucent without any particles after complete lysis. Overnight lysis does not influence the preparation.

   If the sample is lysed in water bath or heating block, vortex occasionally (2 to 3 times per hour) during incubation to lyse readily. Lysis in shaking water bath, shaking incubator or agitator would be best for efficient lysis.

3. ***(Optional :)* If RNA-free DNA is required, add 20 μl of RNase A solution (20 mg/ml, Cat. No. 391-001, not provided), vortex to mix thoroughly, and incubate at room temperature for 2 min.**

   Unless RNase A is treated, RNA will be co-purified with DNA, especially when using transcriptionally active tissues, such as liver and kidney. RNA can inhibit some downstream enzymatic reactions, but will not inhibit PCR itself.

4. **Add 200 μl of Buffer BL to the tube. Vortex the tube to mix thoroughly. Incubate at 70 ˚C for 10 min. Spin down briefly to remove any drops from inside of the lid.**

   Cool down to room temperature before proceeding.

   It is important to mix the sample and Buffer BL thoroughly for good result.

5. **Add 200 μl of absolute ethanol (not provided) to the sample, pulse-vortex to mix the sample thoroughly, and spin down briefly to remove any drops from inside of the lid.**

   It is important to mix the sample and ethanol completely for good result.

   After addition of ethanol, a white precipitate may be formed. It is essential to apply all of the mixture including the precipitate to the Column Type G (mini) on next step.

6. **Transfer all of the mixture to the Column Type G (mini) carefully, centrifuge at 6000 xg above (> 8000 rpm) for 1 min, and replace the collection tube with new one (provided).**
   If the mixture has not passed completely through the membrane, centrifuge again at full speed (> 13000 xg) until all of the solution has passed through. Centrifugation at full speed will not affect DNA recovery.

7. **Add 600 µl of Buffer BW, centrifuge at 6000 xg above (> 8000 rpm) for 1 min and replace the collection tube with new one (provided).**
   If the mini column has colored residue after centrifuge, repeat this step until no colored residue remain. See Trouble shooting guide for detail.
   Centrifugation at full speed (> 13000 xg) will not affect DNA recovery.

8. **Apply 700 µl of Buffer TW. Centrifuge at 6000 xg above (> 8000 rpm) for 1 min. Discard the pass-through and re-insert the mini column back into the collection tube.**
   Centrifugation at full speed will not affect DNA recovery.

9. **Centrifuge at full speed (> 13000 xg) for 1 min to remove residual wash buffer. Place the mini column into a fresh 1.5 ml microcentrifuge tube (not provided).**
   Care must be taken at this step for eliminating the carryover of Buffer TW.
   If a carryover of Buffer TW still occurs, centrifuge again at full speed for 1 min with the collection tube before transferring to the new 1.5 ml microcentrifuge tube.
   Centrifugation must be performed at full speed (13000 to 20000 xg).

10. Add 200 μl of Buffer AE or sterilized water. Incubate at room temperature for 1 min. Centrifuge at full speed (> 13000 xg) for 1 min.

** For the sample expected to yield a little DNA, such as paraffin-embeded, formalin-fixed tissue, or dried blood spot or sperm, it is recommended to use 50 to 150 μl elution buffer as based on the species and conditions of starting sample or the downstream applications.*

Ensure that the Buffer AE or sterilized water is dispensed directly onto the center of mini column membrane for optimal elution of DNA.

Repeat of elution step with fresh 200 μl elution buffer will increase the total DNA yield significantly, while a third elution step with a further 200 μl of elution buffer will increase yields slightly. Each eluate can be separated in fresh tubes or can be collected to same tube, but more than 300 μl of eluate can not be collected in a 1.5 ml microcentrifuge tube because the mini column will come into contact with the eluate.

If higher concentration of DNA is needed or the starting sample amount is very small, the second elution can be carried out with the first eluate instead of fresh elution buffer. Alternatively for higher concentration, the elution volume can be decreased to 50 μl. However the small volume of elution buffer will reduce the total yield of DNA recovery.

For long-term storage, eluting in Buffer AE is recommended. But EDTA included in Buffer AE may inhibit subsequent enzymatic reactions, so you can avoid such latent problem by using distilled deionized water (> pH 7.0) or Tris-HCl (> pH 8.5). *When using water for elution, check the pH of water before elution.*

H PROTOCOL FOR

Paraffin-fixed tissue

Before experiment

- Before first use, add absolute ethanol (ACS grade or better) into Buffer BW and TW as indicated on the bottle.
- Prepare heat block or water bath to 56 °C and 70 °C.
- Prepare 1.5 ml microcentrifuge tube.
- Equilibrate Buffer AE to room temperature.
- All centrifugation should be performed at room temperature.
- Buffer BL and CL may precipitate at cool ambient temperature. If so, dissolve it in 56 °C water bath.

1. **Place a small section of paraffin-fixed tissue (up to 25 mg) in a 2.0 ml microcentrifuge tube (not provided).**
   Minced tissue may be de-paraffinized more efficiently.

2. **Add 1200 µl xylene. Vortex vigorously until the paraffin has been completely melted. Centrifuge at full speed (>13000 xg) for 5 min. Carefully remove supernatant by pipetting.**
   Be careful not to lose any of the pellet.

3. **Add 1200 µl of absolute ethanol (not provided) to the pellet to remove residual xylene and mix by vortexing.**

4. **Centrifuge at full speed for 5 min. Carefully remove the ethanol by pipetting.**
   Do not remove any of the pellet.

5. **Repeat the step 3 and step 4 once or twice.**

6. **Evaporate the residual ethanol by incubating the microcentrifuge tube at room temperature for 10 to 15 min with opened cap.**

7. **Apply 180 µl of Buffer CL and mix completely by vigorous vortexing. Continue with step 2 in <u>G. PROTOCOL FOR Animal tissue</u> on page 21.**

PROTOCOL FOR

Alcohol- or formalin-fixed tissue

Before experiment

- Before first use, add absolute ethanol (ACS grade or better) into Buffer BW and TW as indicated on the bottle.
- Prepare absolute ethanol and 1 X PBS.
- Prepare heat block or water bath to 56 °C and 70 °C.
- Prepare 1.5 ml microcentrifuge tube.
- Equilibrate Buffer AE to room temperature.
- All centrifugation should be performed at room temperature.
- Buffer BL and CL may precipitate at cool ambient temperature. If so, dissolve it in 56 °C water bath.

1. **Briefly blot excess fixative from tissue on clean absorbent paper. Place a small section of fixed tissue (up to 20 mg) in a 1.5 ml microcentrifuge tube (not provided).**
   Minced tissue may be lysed more efficiently.

2. **Apply 400 µl of 1 X PBS to the tube. Vortex to mix, and briefly centrifuge to pellet tissue. Carefully remove supernatant.**
   Remove supernatant by pipetting not to lose the tissue.

3. **Repeat the step 2 once or twice.**

4. **Add 180 µl of Buffer CL. Continue with step 2 in G. PROTOCOL FOR Animal tissue on page 21.**

J PROTOCOL FOR

Dried blood spot

Before experiment

- Before first use, add absolute ethanol (ACS grade or better) into Buffer BW and TW as indicated on the bottle.
- Prepare absolute ethanol.
- Prepare heat block or water bath to 56 °C, 70 °C and 85 °C.
- Prepare 1.5 ml microcentrifuge tube.
- Equilibrate Buffer AE to room temperature.
- All centrifugation should be performed at room temperature.
- Buffer BL and CL may precipitate at cool ambient temperature. If so, dissolve it in 56 °C water bath.

* This protocol is suitable for blood, both untreated and treated with anticoagulants, which has been spotted and dried on filter paper (Schleicher and Schuell 903 or any equivalent).

1. **Place 3 to 4 punched-out circles from a dried blood spot into a 1.5 ml microcentrifuge tube (not provided) and add 200 µl of Buffer CL.**
   Use a 3 mm (1/8") single-hole paper puncher to cut out the circles from a dried blood spot.

2. **Incubate at 85 °C for 10 min. After incubation, spin down the tube briefly to remove any drops from inside of the lid.**
   Do not incubate for more than 15 min.

3. **Add 20 µl of Proteinase K solution (20 mg/ml, provided), vortex to mix, and incubate at 56 °C for 1 hour. Spin down briefly to remove any drops from inside of the lid.**

4. **Add 200 µl of Buffer BL and mix thoroughly by vortexing. Incubate at 70 °C for 10 min. Spin down briefly to remove any drops from inside of the lid.**
   It is essential to mix the sample with Buffer BL completely for efficient lysis.
   After addition of Buffer BL, a white precipitate may be formed. This may be disappeared during incubation at 70 °C and will not affect DNA recovery.

5. **Continue with step 5 in G. PROTOCOL FOR Animal tissue on page 21.**

K PROTOCOL FOR

Gram negative bacteria

Before experiment

- Before first use, add absolute ethanol (ACS grade or better) into Buffer BW and TW as indicated on the bottle.
- Prepare absolute ethanol.
- Prepare heat block or water bath to 56 °C and 70 °C.
- Prepare 1.5 ml microcentrifuge tube.
- Equilibrate Buffer AE to room temperature.
- All centrifugation should be performed at room temperature.
- Buffer CL and BL may precipitate at cool ambient temperature. If so, dissolve it in 56 °C water bath.

1. **Harvest cells (up to 2 x 10^9 cells) in a 1.5 ml microcentrifuge tube (not provided) by centrifugation at full speed for 1 min. Discard supernatant.**
   1 to 2 ml of overnight bacterial culture (A_{600} = 1) may correspond to 1 to 2 x 10^9 cells.

2. **Resuspend the cell pellet thoroughly in 200 µl of Buffer CL.**

3. **Add 20 µl of Proteinase K solution (20 mg/ml, provided). Vortex vigorously to mix completely. Incubate at 56 °C for 15 min.**
   After complete lysis, lysis mixture will turn to clear from turbid. If the lysate still looks turbid or cloudy, incubate until the lysate become clear without any particle.
   Lysis time may vary depending on the species and cell numbers. Cells can be further incubated for complete lysis and longer incubation time does not affect recover yield.
   After incubation, cool the lysate to room temperature.

4. **Spin down the tube briefly to remove any drops from inside of the lid.**

5. **Continue with step 3 in G. PROTOCOL FOR Animal tissue on page 21.**

PROTOCOL FOR
Gram positive bacteria

Before experiment

- Before first use, add absolute ethanol (ACS grade or better) into Buffer BW and TW as indicated on the bottle.
- Prepare heat block or water bath to 37 °C, 56 °C and 70 °C.
- Prepare Lysozyme (LYS702, Bioshop, Canada, or equivalent) or L ysostaphin (L7386, SIGMA, USA, or equivalent).
- Prepare 1.5 ml microcentrifuge tube.
- Prepare absolute ethanol.
- Equilibrate Buffer AE to room temperature.
- All centrifugation should be performed at room temperature.
- Buffer BL and CL may precipitate at cool ambient temperature. If so, dissolve it in 56 °C water bath.

- **Prepare Enzyme Mixture;** Resuspend the appropriate enzyme (not provided, listed below) with Buffer GP just before use. Enzyme mixture should be stored at -20°C (or below) as small aliquots; ideally, once per an aliquot. Thawed aliquot should be discarded.

  30 mg/ml lysozyme (LYS702, Bioshop, Canada, or equivalent)
  or/and
  300 μg/ml lysostaphin (L9043, SIGMA, USA, or equivalent)

  * For certain species, such as *Staphylococcus*, treatment of lysostaphin (final conc. = 300 μg/ml) may be required for efficient lysis instead of (or with) lysozyme. However, lysozyme is sufficient to lyse the cell wall for most gram positive bacterial strains.

1. **Harvest cells (up to 2 x 10^9 cells) in a 1.5 ml microcentrifuge tube (not provided) by centrifugation at full speed for 1 min. Discard supernatant.**

2. **Resuspend the cell pellet thoroughly in 180 μl of the prepared enzyme mixture. Incubate at 37 °C for 30 min. After incubation, spin down the tube briefly to remove any drops from inside of the lid.**
   The purpose of this treatment is to weaken the cell wall so that efficient cell lysis can take place.

3. ***(Optional :)* If RNA-free DNA is required, add 20 μl of RNase A solution (20 mg/ml, Cat. No. 391-001, not provided) to the sample, mix well by vortexing and incubate at room temperature for 2 min.**
   Unless RNase A is treated, RNA will be copurified with DNA. RNA may inhibit some downstream enzymatic reactions, but will not inhibit PCR itself.

4. **Add 20 μl of Proteinase K solution (20 mg/ml, provided) and 200 μl of Buffer BL. Mix completely by vigorous vortexing or pipetting.**

5. **Incubate at 56 °C for 30 min and then at 70 °C for a further 30 min.**
   If any pathogen is subjected, it is strongly recommended that additional incubation at 70 °C for 30 min should be substituted by at 95 °C for 15 min.
   Longer incubation at 95 °C will degrade DNA.
   After incubation, cool to room temperature.

6. **Spin down the tube briefly to remove any drops from inside of the lid.**

7. **Continue with step 5 in G. PROTOCOL FOR Animal tissue on page 21.**

M PROTOCOL FOR

0.4 to 1 ml of whole blood

Before experiment

- Before first use, add absolute ethanol (ACS grade or better) into Buffer BW and TW as indicated on the bottle.
- Prepare heat block or water bath to 56 °C.
- Prepare 15 ml microcentrifuge tube.
- Prepare absolute ethanol.
- Equilibrate Buffer AE to room temperature.
- All centrifugation should be performed at room temperature.
- Buffer BL and CL may precipitate at cool ambient temperature. If so, dissolve it in 56 °C water bath.

1. **Pipet 50 µl of Proteinase K solution (20 mg/ml, provided) into the bottom of a 15 ml conical tube (not provided).**

2. **Add 1 ml of the sample to the tube and mix well.**
   If the sample volume is less than 1 ml, adjust the volume to 1 ml with 1 X PBS.

3. **Add 1.2 ml of Buffer BL to the tube. Vortex the tube for 15 s to mix thoroughly.**
   For efficient lysis and consistent result, it is essential to mix the sample completely.

4. **Incubate at 56 °C for 20 min. After incubation, spin down the tube briefly to remove any drops from inside of the lid.**
   During incubation, occasional vortexing of the lysate will help accelerate lysis.Longer incubation will not affect DNA recovery.

5. **Add 1 ml of absolute ethanol (not provided) to the sample, pulse-vortex to mix the sample thoroughly, and spin down briefly to remove any drops from inside of the lid.**
   It is essential to mix the sample completely for efficient binding.

6. **Transfer all of the mixture to a midi column carefully, close the cap, centrifuge at 2000 xg (3000 rpm) for 3 min.**
   While transfer of the mixture to a midi column, be careful not to moisten the rim of a midi column.If the mixture has not passed completely through the membrane, centrifuge again at higher speed until all of the solution has passed through.

7. **Discard the filtrate and re-insert the midi column back into the 15 ml tube. Apply 3 ml of Buffer BW and centrifuge at 2000 xg (3000 rpm) for 3 min.**
   Wipe off any spillage from the thread of the 15 ml tube before re-inserting the midi column.

8. **Discard the filtrate and re-insert the midi column back into the 15 ml tube. Apply 3 ml of Buffer TW and centrifuge at 4500 xg (5000 rpm) for 3 min.**
   If the column membrane has residual ethanol (originated or Buffer TW) associated with it after centrifugation, incubate the midi column at room temperature for 15 min to evaporate residual ethanol.The residual ethanol can decrease DNA yield significantly and it also can inhibit some downstream applications.

9. **(Optional :) If the centrifugal force applied at previous step is less than 4500 xg, follow these;**
   → ***Discard the filtrate, wipe off any spillage from the thread of the 15 ml tube, and re-insert the midi column back into the 15 ml tube. Apply 1 ml of absolute ethanol and centrifuge for additional 15 min at available full speed. Remove the midi column and incubate it at room temperature for 15 min.***
   Insufficient centrifugal force will bring on residual ethanol in midi column membrane, followed by poor DNA recovery. At least, 4000 xg is required for proper DNA recovery.

10. **Place the midi column into a new 15 ml conical tube (provided). Pipet 300 μl of Buffer AE or distilled water onto a center of membrane and close the cap. Incubate at room temperature for 5 min. Centrifuge for at 4500 xg (5000 rpm) for 5 min.**
    Before this elution step, it is strongly recommended that any residual ethanol originated from Buffer TW should not remain in midi column membrane. It can be checked by smell. Residual ethanol disturbs proper DNA preparation as follows; poor DNA recovery, low purity, inhibition of subsequent reactions, and etc.
    Ensure that the Buffer AE or distilled water is dispensed directly onto the center of the midi column membrane for optimal elution of DNA.
    Less than 300 μl of eluate will be obtained from 300 μl of elution buffer, but this has no influence on DNA yields.
    If the sample weight, the amount of cell, or sample volume is small, less volume of Buffer AE or distilled water can be applied. However, do not reduce the elution volume below 100 μl.
    For long-term storage, eluting in Buffer AE is recommended. But, EDTA included in the Buffer AE may inhibit subsequent enzymatic reactions, so you can avoid such latent problems by using distilled water (> pH 7.0) or Tris-HCl (> pH 8.5). when using water for elution, make sure the pH of water is higher than 7.0.

11. **For higher concentrated yield, re-load the eluate from step 10 into the midi column, close the cap, incubate at room temperature for 5 min, and centrifuge at 4500 xg (5000 rpm) for 5 min.**
Less than 300 μl of eluate will be obtained from 300 μl of elution buffer, but this has no influence on DNA yields.

For higher total yield, add 300 μl of fresh Buffer AE or distilled water again into the midi column, close the cap, incubate at room temperature for 5 min, and centrifuge at 4500 xg (5000 rpm) for 5 min.
The first and second eluates can be combined or collected separately as necessity. Less than 300 μl of eluate will be obtain from 300 μl of elution buffer, but this has no influence on DNA yields.

N PROTOCOL FOR

1 to 2 ml of whole blood

Before experiment

- Before first use, add absolute ethanol (ACS grade or better) into Buffer BW and TW as indicated on the bottle.
- Prepare heat block or water bath to 56 °C.
- Prepare 15 ml microcentrifuge tube.
- Prepare absolute ethanol.
- Equilibrate Buffer AE to room temperature.
- All centrifugation should be performed at room temperature.
- Buffer BL and CL may precipitate at cool ambient temperature. If so, dissolve it in 56 °C water bath.

1. **Pipet 100 μl of Proteinase K solution (20 mg/ml, provided) into the bottom of a 15 ml conical tube (not provided).**

2. **Add 2 ml of the sample to the tube and mix well.**
   If the sample volume is less than 2 ml, adjust the volume to 2 ml with 1 X PBS.

3. **Add 2.4 ml of Buffer BL to the tube. Vortex the tube for 15 s to mix thoroughly.**
   For efficient lysis and consistent result, it is essential to mix the sample completely.

4. **Incubate at 56 °C for 20 min. After incubation, spin down the tube briefly to remove any drops from inside of the lid.**
   During incubation, occasional vortexing of the lysate will help accelerate lysis. Longer incubation will not affect DNA recovery.

5. **Add 2 ml of absolute ethanol (not provided) to the sample, pulse-vortex to mix the sample thoroughly, and spin down briefly to remove any drops from inside of the lid.**
   It is essential to mix the sample completely for efficient binding.

6. **Transfer 4 ml of the mixture to a midi column carefully, close the cap, centrifuge at 2000 xg (3000 rpm) for 3 min.**
   While transfer of the mixture to a midi column, be careful not to moisten the rim of a midi column. If the mixture has not passed completely through the membrane, centrifuge again at higher speed until all of the solution has passed through.

7. **Discard the filtrate and re-insert the midi column back into the 15 ml tube. Apply the remainder of the mixture, close the cap, and centrifuge at 2000 xg (3000 rpm) for 3 min.**
   Wipe off any spillage from the thread of the 15 ml tube before re-inserting the midi column. While transfer of the mixture to the midi column, be careful not to moisten the rim of midi column. If the mixture has not passed completely through the membrane, centrifuge again at higher speed until all of the solution has passed through.

8. **Discard the filtrate and re-insert the midi column back into the 15 ml tube. Apply 3 ml of Buffer BW and centrifuge at 2000 xg (3000 rpm) for 3 min.**
   Wipe off any spillage from the thread of the 15 ml tube before re-inserting the midi column.

9. **Discard the filtrate and re-insert the midi column back into the 15 ml tube. Apply 3 ml of Buffer TW and centrifuge at 4500 xg (5000 rpm) for 3 min.**
   If the column membrane has residual ethanol (originated or Buffer TW) associated with it after centrifugation, incubate the midi column at room temperature for 15 min to evaporate residual ethanol. The residual ethanol can decrease DNA yield significantly and it also can inhibit some downstream applications.

10. **(Optional :) If the centrifugal force applied at previous step is less than 4500 xg, follow these;**
    → ***Discard the filtrate, wipe off any spillage from the thread of the 15 ml tube, and re-insert the midi column back into the 15 ml tube. Apply 1 ml of absolute ethanol and centrifuge for additional 15 min at available full speed. Remove the midi column and incubate it at room temperature for 15 min.***
    Insufficient centrifugal force will bring on residual ethanol in midi column membrane, followed by poor DNA recovery. At least, 4000 xg is required for proper DNA recovery.

11. **Place the midi column into a new 15 ml conical tube (provided). Pipet 400 μl of Buffer AE or distilled water onto a center of membrane and close the cap. Incubate at room temperature for 5 min. Centrifuge for at 4500 xg (5000 rpm) for 5 min.**

    Before this elution step, it is strongly recommended that any residual ethanol originated from Buffer TW should not remain in midi column membrane. It can be checked by smell. Residual ethanol disturbs proper DNA preparation as follows; poor DNA recovery, low purity, inhibition of subsequent reactions, and etc.

    Ensure that the Buffer AE or distilled water is dispensed directly onto the center of the midi column membrane for optimal elution of DNA.

    Less than 400 μl of eluate will be obtained from 400 μl of elution buffer, but this has no influence on DNA yields.

    If the sample weight, the amount of cell, or sample volume is small, less volume of Buffer AE or distilled water can be applied. However, do not reduce the elution volume below 100 μl.

    For long-term storage, eluting in Buffer AE is recommended. But, EDTA included in the Buffer AE may inhibit subsequent enzymatic reactions, so you can avoid such latent problems by using distilled water (> pH 7.0) or Tris-HCl (> pH 8.5). When using water for elution, make sure the pH of water is higher than 7.0.

12. **For higher concentrated yield, re-load the eluate from step 11 into the midi column, close the cap, incubate at room temperature for 5 min, and centrifuge at 4500 xg (5000 rpm) for 5 min.**

    Less than 400 μl of eluate will be obtained from 400 μl of elution buffer, but this has no influence on DNA yields.

    For higher total yield, add 400 μl of fresh Buffer AE or distilled water again into the midi column, close the cap, incubate at room temperature for 5 min, and centrifuge at 4500 xg (5000 rpm) for 5 min.

    The first and second eluates can be combined or collected separately as necessity. Less than 400 μl of eluate will be obtain from 400 μl of elution buffer, but this has no influence on DNA yields.

O PROTOCOL FOR

2 x 10^7 of Cultured cells

Before experiment

- Before first use, add absolute ethanol (ACS grade or better) into Buffer BW and TW as indicated on the bottle.
- Prepare heat block or water bath to 56 °C and 70 °C.
- Prepare 15 ml microcentrifuge tube.
- Prepare absolute ethanol.
- Equilibrate Buffer AE to room temperature.
- All centrifugation should be performed at room temperature.
- Buffer BL and CL may precipitate at cool ambient temperature. If so, dissolve it in 56 °C water bath.

1. **Pellet cells (up to 2 x 10^7 cells) to a 15 ml conical tube (not provided) by centrifugation at 2000 xg for 5 min.**
   Certain cell strains, such as PCI 2, are not lysis well in Buffer CL. For those cells, it is helpful to perform additional freeze-thaw step several times before proceeding to next step.

2. **Discard the supernatant as much as possible and re-suspend thoroughly cell pellet in 1 ml of Buffer CL.**
   Pelleted cells may not be re-suspended easily in Buffer CL. It is helpful to re-suspended the cell pellet with residual media by flickering or vortexing before the addition of Buffer CL.

3. **Add 100 µl of Proteinase K solution (20 mg/ml, provided). Mix thoroughly by vortexing. Incubate at 56 °C for 20 min. After incubation, spin down the tube briefly to remove any drops from inside of the lid.**
   Vortex the lysate occasionally to accelerate during incubation. Longer incubation will not affect DNA recovery.

4. **(Optional :) If RNA-free DNA is required, cool the mixture to room temperature, add 20 µl of RNase A solution (100 mg/ml, Cat. No. 117-961, not provided), vortex to mix thoroughly, and incubate for 5 min at room temperature.**
   Unless RNase A is treated, both DNA and RNA will be co-purified. RNA can inhibit some downstream enzymatic reactions, but not PCR itself.

5. **Add 1.2 ml of Buffer BL to the tube. Vortex the tube for 15 s to mix thoroughly.**
   For efficient lysis and consistent result, it is essential to mix the sample completely.

6. **Incubate at 70 ˚C for 10 min. After incubation, spin down the tube briefly to remove any drops from inside of the lid.**

7. **Continue with step 5 in M. PROTOCOL FOR 0.4 to 1 ml of whole blood on page 31.**

PROTOCOL FOR

30 to 100 mg of animal tissue

Before experiment

- Before first use, add absolute ethanol (ACS grade or better) into Buffer BW and TW as indicated on the bottle.
- Prepare heat block or water bath to 56 °C and 70 °C.
- Prepare 15 ml microcentrifuge tube.
- Prepare absolute ethanol.
- Equilibrate Buffer AE to room temperature.
- All centrifugation should be performed at room temperature.
- Buffer BL and CL may precipitate at cool ambient temperature. If so, dissolve it in 56 °C water bath.

1. Homogenize 30 to 100 mg of tissue as described in step 1a, 1b, or 1c, depending on the sample type.

It is most important to weight the sample accurately.If the sample is spleen tissue, up to 40 mg can be processed. Well-disrupted sample will accelerate lysis and decrease the lysis time.

1a. For soft tissue, such as liver or brain, put up to 100 mg of the tissue into a 15 ml conical tube (not provided), add 400 µl of Buffer CL, homogenize thoroughly on ice with homogenizer, add 600 µl of Buffer CL, and vortex vigorously to homogenate well.

1b. If a homogenizer is not available or the tissue is not soft, grind the tissue to a fine powder with liquid nitrogen in a pre-cooled mortar and pestle. Put up to 100 mg of the powdered tissue into a 15 ml conical tube (not provided). Add 1 ml of Buffer CL and vortex for 30 s to homogenate completely.

1c. If neither 1a nor 1b is available, mince the tissue with sterile sharp blade or scalpel as small as possible. Put up to 100 mg of the tissue into a 15 ml conical tube (not provided). Add 1 ml of Buffer CL and pulse vortex for 30 s.

** ***Alternatively, tissue sample can be effectively disrupted using some instruments, such as rotor-stator homogenizer or a bead-beater. When use these, follow the manufacture's instruction manual.***

2. **Add 100 μl of Proteinase K solution (20 mg/ml) to the tube. Mix completely by vortexing or pipetting.Incubate at 56 ˚C until the sample is completely lysed. After incubation, spin down the tube briefly to remove any drops from inside of the lid.**
   It is essential to mix the components completely for efficient lysis.Lysis time varied from 10 min to 3 hours usually depending on the type of sample and homogenization method. The lysate should become translucent without any particle after complete lysis. Overnight incubation does not influence the preparation. If the sample is lysed in water bath or heating block, vortex occasionally (2 to 3 times per hour) during incubation to lyse readily. Lysis in shaking incubator, or agitator would be best for efficient lysis.

3. **(Optional :) If RNA-free DNA is required, cool the mixture to room temperature, add 20 μl of RNase A solution (100 mg/ml, Cat. No. 117-961, not provided), vortex to mix thoroughly, and incubate for 3 min at room temperature.**
   Unless RNase A is treated, both DNA and RNA will be co-purified. RNA can inhibit some downstream enzymatic reactions, but not PCR itself.

4. **Add 1.2 ml of Buffer BL to the tube. Vortex the tube for 15 s to mix thoroughly.**
   For efficient lysis and consistent result, it is essential to mix the sample completely.

5. **Incubate at 70 ˚C for 10 min. After incubation, spin down the tube briefly to remove any drops from inside of the lid.**

6. **Continue with step 5 in M. PROTOCOL FOR 0.4 to 1 ml of whole blood on page 31.**

Q PROTOCOL FOR

Up to 1 x 10^{10} of gram negative bacteria

Before experiment

- Before first use, add absolute ethanol (ACS grade or better) into Buffer BW and TW as indicated on the bottle.
- Prepare heat block or water bath to 56 °C and 70 °C.
- Prepare 15 ml microcentrifuge tube.
- Prepare absolute ethanol.
- Equilibrate Buffer AE to room temperature.
- All centrifugation should be performed at room temperature.
- Buffer BL and CL may precipitate at cool ambient temperature. If so, dissolve it in 56 °C water bath.

1. **Harvest bacterial cells (up to 1 x 10^{10}) in a 15 ml conical tube by centrifugation at 10000 xg for 5 min. Discard the supernatant as much as possible.**
   The number of cells in bacterial culture varies depending on each strain. When A_{600} = 1, 10 ml of bacterial culture may correspond to 1 x 10^{10} to 2 x 10^{10} cells approximately.

2. **Re-suspend the cell pellet with the residual liquid by flickering or vortexing.**

3. **Add 1 ml of Buffer CL and re-suspend completely by pipetting or vortexing.**

4. **Add 50 μl of Proteinase K solution (20 mg/ml, provided) to the tube. Mix completely by vortexing or pipetting.**

5. **Incubate at 56 °C for 20 min. After incubation, spin down the tube briefly to remove any drops from inside of the lid.**
   Vortex the lysate occasionally during incubation to accelerate lysis. Longer incubation will not affect DNA recovery.

6. **(Optional :) If RNA-free DNA is required, cool the mixture to room temperature, add 20 μl of RNase A solution (100 mg/ml, Cat. No. 117-961, not provided), vortex to mix thoroughly, and incubate for 3 min at room temperature.**
   Unless RNase A is treated, both DNA and RNA will be co-purified. RNA can inhibit some downstream enzymatic reactions, but not PCR itself.

7. **Add 1.2 ml of Buffer BL to the tube. Vortex the tube for 15 s to mix thoroughly.**
   For efficient lysis and consistent result, it is essential to mix the sample completely.

8. **Incubate at 70 ˚C for 10 min. After incubation, spin down the tube briefly to remove any drops from inside of the lid.**

9. **Continue with step 5 in M. PROTOCOL FOR 0.4 to 1 ml of whole blood on page 31.**

R PROTOCOL FOR

3 to 5 ml of whole blood

Before experiment

- Before first use, add absolute ethanol (ACS grade or better) into Buffer BW and TW as indicated on the bottle.
- Prepare heat block or water bath to 65 °C.
- Prepare 50 ml conical tube.
- Prepare absolute ethanol.
- Equilibrate Buffer AE to room temperature.
- All centrifugation should be performed at room temperature.
- Buffer BL and CL may precipitate at cool ambient temperature. If so, dissolve it in 56 °C water bath.

1. **Pipet 200 μl of Proteinase K solution (20 mg/ml, provided) into the bottom of a 50 ml conical tube (not provided).**

2. **Add 5 ml of the sample to the tube and mix well.**
   If the sample volume is less than 5 ml, adjust the volume to 5 ml with 1 X PBS.

3. **Add 6 ml of Buffer BL to the tube. Vortex the tube for 15 s to mix thoroughly.**
   For efficient lysis and consistent result, it is essential to mix the sample completely.

4. **Incubate at 65 °C for 20 min. After incubation, spin down the tube briefly to remove any drops from inside of the lid.**
   During incubation, occasional vortexing of the lysate will help accelerate lysis. Longer incubation will not affect DNA recovery.

5. **Add 5 ml of absolute ethanol (not provided) to the sample, pulse-vortex to mix the sample thoroughly, and spin down briefly to remove any drops from inside of the lid.**
   It is essential to mix the sample completely for efficient binding.

6. **Transfer all of the mixture to a maxi column carefully, close the cap, centrifuge at 2000 xg (3000 rpm) for 3 min.**
   While transfer of the mixture to a maxi column, be careful not to moisten the rim of a maxi column. DO NOT place the maxi column in tilted or bottom-up position even if caps are closed.If the mixture has not passed completely through the membrane, centrifuge again at higher speed until all of the solution has passed through.

7. **Discard the filtrate and re-insert the maxi column back into the 50 ml tube. Apply 7 ml of Buffer BW and centrifuge at 2000 xg (3000 rpm) for 3 min.**
   Wipe off any spillage from the thread of the 50 ml tube before re-inserting the maxi column.

8. **Discard the filtrate and re-insert the maxi column back into the 50 ml tube. Apply 10 ml of Buffer TW and centrifuge at 4500 xg (5000 rpm) for 15 min.**
   If the column membrane has residual ethanol (originated or Buffer TW) associated with it after centrifugation, incubate the maxi column at room temperature for 15 min to evaporate residual ethanol.The residual ethanol can decrease DNA yield significantly and it also can inhibit some downstream applications.

9. **(Optional :) If the centrifugal force applied at previous step is less than 4500 xg, follow these;**
   → ***Discard the filtrate, wipe off any spillage from the thread of the 50 ml tube, and re-insert the maxi column back into the 50 ml tube. Apply 3 ml of absolute ethanol and centrifuge for additional 15 min at available full speed. Remove the maxi column and incubate it at room temperature for 20 min.***
   Insufficient centrifugal force will bring on residual ethanol in maxi column membrane, followed by poor DNA recovery. At least, 4000 xg is required for proper DNA recovery.

10. **Place the maxi column into a new 50 ml conical tube (provided). Pipet 600 μl of Buffer AE or distilled water onto a center of membrane and close the cap. Incubate at room temperature for 5 min. Centrifuge at 4500 xg (5000 rpm) for 5 min.**

    Before this elution step, it is strongly recommended that any residual ethanol originated from Buffer TW should not remain in maxi column membrane. It can be checked by smell. Residual ethanol disturbs proper DNA preparation as follows; poor DNA recovery, low purity, inhibition of subsequent reactions, and etc.

    Ensure that the Buffer AE or distilled water is dispensed directly onto the center of the maxi column membrane for optimal elution of DNA. Less than 600 μl of eluate will be obtained from 600 μl of elution buffer, but this has no influence on DNA yields.

    If the sample weight, the amount of cell, or sample volume is small, less volume of Buffer AE or distilled water can be applied. However, do not reduce the elution volume below 300 μl.

    For long-term storage, eluting in Buffer AE is recommended. But, EDTA included in the Buffer AE may inhibit subsequent enzymatic reactions, so you can avoid such latent problems by using distilled water (> pH 7.0) or Tris-HCl (> pH 8.5). when using water for elution, make sure the pH of water is higher than 7.0.

11. **For higher concentrated yield, re-load the eluate from step 10 into the maxi column, close the cap, incubate at room temperature for 5 min, and centrifuge at 4500 xg (5000 rpm) for 5 min.**

    Less than 600 μl of eluate will be obtained from 600 μl of elution buffer, but this has no influence on DNA yields.

    For higher total yield, add 600 μl of fresh Buffer AE or distilled water again into the maxi column, close the cap, incubate at room temperature for 5 min, and centrifuge at 4500 xg (5000 rpm) for 5 min.

    The first and second eluates can be combined or collected separately as necessity. Less than 600 μl of eluate will be obtain from 600 μl of elution buffer, but this has no influence on DNA yields.

S PROTOCOL FOR

6 to 10 ml of whole blood

Before experiment

- Before first use, add absolute ethanol (ACS grade or better) into Buffer BW and TW as indicated on the bottle.
- Prepare heat block or water bath to 65 °C.
- Prepare 50 ml conical tube.
- Prepare absolute ethanol.
- Equilibrate Buffer AE to room temperature.
- All centrifugation should be performed at room temperature.
- Buffer BL and CL may precipitate at cool ambient temperature. If so, dissolve it in 56 °C water bath.

1. **Pipet 400 µl of Proteinase K solution (20 mg/ml, provided) into the bottom of a 50 ml conical tube (not provided).**

2. **Add 10 ml of the sample to the tube and mix well.**
   If the sample volume is less than 5 ml, adjust the volume to 10 ml with 1 X PBS.

3. **Add 12 ml of Buffer BL to the tube. Vortex the tube for 15 s to mix thoroughly.**
   For efficient lysis and consistent result, it is essential to mix the sample completely.

4. **Incubate at 65 °C for 20 min. After incubation, spin down the tube briefly to remove any drops from inside of the lid.**
   During incubation, occasional vortexing of the lysate will help accelerate lysis. Longer incubation will not affect DNA recovery.

5. **Add 10 ml of absolute ethanol (not provided) to the sample, pulse-vortex to mix the sample thoroughly, and spin down briefly to remove any drops from inside of the lid.**
   It is essential to mix the sample completely for efficient binding.

6. **Transfer a half of the mixture to a maxi column carefully, close the cap, centrifuge at 2000 xg (3000 rpm) for 3 min.**
   While transfer of the mixture to a maxi column, be careful not to moisten the rim of a maxi column. DO NOT place the maxi column in tilted or bottom-up position even if caps are closed. If the mixture has not passed completely through the membrane, centrifuge again at higher speed until all of the solution has passed through.

7. **Discard the filtrate and re-insert the maxi column back into the 50 ml tube. Apply the remainder of the mixture, close the cap, and centrifuge at 2000 xg (3000 rpm) for 3 min.**
   Wipe off any spillage from the thread of the 50 ml tube before re-inserting the maxi column. While transfer of the mixture to the maxi column, be careful not to moisten the rim of maxi column.If the mixture has not passed completely through the membrane, centrifuge again at higher speed until all of the solution has passed through.

8. **Discard the filtrate and re-insert the maxi column back into the 50 ml tube. Apply 7 ml of Buffer BW and centrifuge at 2000 xg (3000 rpm) for 3 min.**
   Wipe off any spillage from the thread of the 50 ml tube before re-inserting the maxi column.

9. **Discard the filtrate and re-insert the maxi column back into the 50 ml tube. Apply 10 ml of Buffer TW and centrifuge at 4500 xg (5000 rpm) for 15 min.**
   If the column membrane has residual ethanol (originated or Buffer TW) associated with it after centrifugation, incubate the maxi column at room temperature for 15 min to evaporate residual ethanol.The residual ethanol can decrease DNA yield significantly and it also can inhibit some downstream applications.

10. **(Optional :) If the centrifugal force applied at previous step is less than 4500 xg, follow these;**
    → ***Discard the filtrate, wipe off any spillage from the thread of the 50 ml tube, and re-insert the maxi column back into the 50 ml tube. Apply 3 ml of absolute ethanol and centrifuge for additional 15 min at available full speed. Remove the maxi column and incubate it at room temperature for 20 min.***
    Insufficient centrifugal force will bring on residual ethanol in maxi column membrane, followed by poor DNA recovery. At least, 4000 xg is required for proper DNA recovery.

11. **Place the maxi column into a new 50 ml conical tube (provided). Pipet 600 μl of Buffer AE or distilled water onto a center of membrane and close the cap. Incubate at room temperature for 5 min. Centrifuge at 4500 xg (5000 rpm) for 5 min.**

    Before this elution step, it is strongly recommended that any residual ethanol originated from Buffer TW should not remain in maxi column membrane. It can be checked by smell. Residual ethanol disturbs proper DNA preparation as follows; poor DNA recovery, low purity, inhibition of subsequent reactions, and etc.

    Ensure that the Buffer AE or distilled water is dispensed directly onto the center of the maxi column membrane for optimal elution of DNA. Less than 1 ml of eluate will be obtained from 1 ml of elution buffer, but this has no influence on DNA yields.

    If the volume of starting sample is less than 10 ml, less volume of Buffer AE or distilled water can be applied. However, do not reduce the elution volume below 300 μl.

    For long-term storage, eluting in Buffer AE is recommended. But, EDTA included in the Buffer AE may inhibit subsequent enzymatic reactions, so you can avoid such latent problems by using distilled water (> pH 7.0) or Tris-HCl (> pH 8.5). when using water for elution, make sure the pH of water is higher than 7.0.

12. **For higher concentrated yield, re-load the eluate from step 11 into the maxi column, close the cap, incubate at room temperature for 5 min, and centrifuge at 4500 xg (5000 rpm) for 5 min.**

    Less than 1 ml of eluate will be obtained from 1 ml of elution buffer, but this has no influence on DNA yields.

    For higher total yield, add 1 ml of fresh Buffer AE or distilled water again into the maxi column, close the cap, incubate at room temperature for 5 min, and centrifuge at 4500 xg (5000 rpm) for 5 min.

    The first and second eluates can be combined or collected separately as necessity.

    Less than 1 ml of eluate will be obtain from 1 ml of elution buffer, but this has no influence on DNA yields.

PROTOCOL FOR

Up to 1 x 10^8 of cultured cells

Before experiment

- Before first use, add absolute ethanol (ACS grade or better) into Buffer BW and TW as indicated on the bottle.
- Prepare heat block or water bath to 65 °C abd 70 °C.
- Prepare 50 ml conical tube.
- Prepare absolute ethanol.
- Equilibrate Buffer AE to room temperature.
- All centrifugation should be performed at room temperature.
- Buffer BL and CL may precipitate at cool ambient temperature. If so, dissolve it in 56 °C water bath.

1. **Pellet cells (up to 1 x 10^8 cells) to a 50 ml conical tube (not provided) by centrifugation at 2000 xg for 5 min.**
   Certain cell strains, such as PCI 2, are not lysed well in Buffer CL. For those cells, it is helpful to perform additional free-thaw step several times before proceeding to next step.

2. **Discard the supernatants as much as possible and re-suspend thoroughly cell pellet in 5 ml of Buffer CL.**
   Pelleted cells may not be re-suspended easily in Buffer CL. It is helpful to re-suspend the cell pellet with residual media by flickering or vortexing before the addition of Buffer CL.

3. **Add 200 µl of Proteinase K solution (20 mg/ml, provided). Mix thoroughly by vortexing. Incubate at 65 °C for 20 min. After incubation, spin down the tube briefly to remove any drops from inside of the lid.**
   Vortex the lysate occasionally to accelerate during incubation. Longer incubation will not affect DNA recovery.

4. **(Optional :) If RNA-free DNA is required, cool the mixture to room temperature, add 100 µl of RNase A solution (100 mg/ml, Cat. No. 117-961, not provided) and vortex to mix thoroughly. Incubate at room temperature for 5 min.**
   Unless RNase A is treated, both DNA and RNA will be co-purified. RNA can inhibit some downstream enzymatic reactions, but not PCR itself.

U PROTOCOL FOR

100 to 250 mg of animal tissue

Before experiment

- Before first use, add absolute ethanol (ACS grade or better) into Buffer BW and TW as indicated on the bottle.
- Prepare heat block or water bath to 56 °C abd 70 °C.
- Prepare 50 ml conical tube.
- Prepare absolute ethanol.
- Equilibrate Buffer AE to room temperature.
- All centrifugation should be performed at room temperature.
- Buffer BL and CL may precipitate at cool ambient temperature. If so, dissolve it in 56 °C water bath.

1. Homogenize 100 to 250 mg of tissue as described in step 1a, 1b, or 1c, depending on the sample type.

It is most important to weight the sample accurately. If the sample is spleen tissue, up to 100 mg can be processed. Well-disrupted sample will accelerate lysis and decrease the lysis time.

1a. For soft tissue, such as liver or brain, put up to 250 mg of the tissue into homogenizer, add 1 ml of distilled water, homogenize thoroughly on ice. Transfer the homogenate into a 50 ml conical tube (not provided), add 3 ml of Buffer CL, and vortex vigorously to homogenate well.

1b. If a homogenizer is not available or the tissue is not soft, grind the tissue to a fine powder with liquid nitrogen in a pre-cooled mortar and pestle. Put up to 250 mg of the powdered tissue into a 50 ml conical tube (not provided). Add 4 ml of Buffer CL and vortex for 30 s to homogenate completely.

1c. If neither 1a nor 1b is available, mince the tissue with sterile sharp blade or scalpel as small as possible. Put up to 100 mg of the tissue into a 15 ml conical tube (not provided). Add 4 ml of Buffer CL and pulse vortex for 30 s.

** ***Alternatively, tissue sample can be effectively disrupted using some instruments, such as rotor-stator homogenizer or a bead-beater. When use these, follow the manufacture's instruction manual.***

2. **Add 200 μl of Proteinase K solution (20 mg/ml) to the tube. Mix completely by vortexing or pipetting. Incubate at 56 °C until the sample is completely lysed. After incubation, spin down the tube briefly to remove any drops from inside of the lid.**
   It is essential to mix the components completely for efficient lysis.Lysis time varied from 10 min to 3 hours usually depending on the type of sample and homogenization method. The lysate should become translucent without any particle after complete lysis. Overnight incubation does not influence the preparation. If the sample is lysed in water bath or heating block, vortex occasionally (2 to 3 times per hour) during incubation to lyse readily. Lysis in shaking incubator, or agitator would be best for efficient lysis.

3. **(Optional :) If RNA-free DNA is required, cool the mixture to room temperature, add 20 μl of RNase A solution (100 mg/ml, Cat. No. 117-961, not provided), vortex to mix thoroughly, and incubate for 3 min at room temperature.**
   Unless RNase A is treated, both DNA and RNA will be co-purified. RNA can inhibit some downstream enzymatic reactions, but not PCR itself.

4. **Add 5 ml of Buffer BL to the tube. Vortex the tube for 15 s to mix thoroughly.**
   For efficient lysis and consistent result, it is essential to mix the sample completely.

5. **Incubate at 70 °C for 10 min. After incubation, spin down the tube briefly to remove any drops from inside of the lid.**

6. **Add 4 ml of absolute ethanol (not provided) to the sample, vortex to mix the sample thoroughly.**
   It is essential to mix the sample completely for efficient binding.A white thread-like strands can be formed in the lysate. It is essential to transfer all of the lysate including this to a maxi column at next step.

7. **Continue with step 6 in R. PROTOCOL FOR 3 to 5 ml of whole blood on page 43.**

V PROTOCOL FOR

Up to 5 x 10^{10} of gram negative bacteria

Before experiment

- Before first use, add absolute ethanol (ACS grade or better) into Buffer BW and TW as indicated on the bottle.
- Prepare heat block or water bath to 56 °C abd 70 °C.
- Prepare 50 ml conical tube.
- Prepare absolute ethanol.
- Equilibrate Buffer AE to room temperature.
- All centrifugation should be performed at room temperature.
- Buffer BL and CL may precipitate at cool ambient temperature. If so, dissolve it in 56 °C water bath.

1. **Harvest bacterial cells (up to 5 x 10^{10}) in a 50 ml conical tube by centrifugation at 10000 xg for 5 min. Discard the supernatant as much as possibleThe number of cells in bacterial culture varies depending on each strain. When A_{600} = 1, 10 ml of bacterial culture may correspond to 1 x 10^{10} to 2 x 10^{10} cells approximately.**

2. **Re-suspend the cell pellet with the residual liquid by flickering or vortexing.**

3. **Add 5 ml of Buffer CL and re-suspend completely by pipetting or vortexing.**

4. **Add 200 μl of Proteinase K solution (20 mg/ml, provided) to the tube. Mix completely by vortexing or pipetting.**

5. **Incubate at 56 °C for 30 min. After incubation, spin down the tube briefly to remove any drops from inside of the lid.**
   Vortex the lysate occasionally during incubation to accelerate lysis. Longer incubation will not affect DNA recovery.

6. **(Optional :) If RNA-free DNA is required, cool the mixture to room temperature, add 20 μl of RNase A solution (100 mg/ml, Cat. No. 117-961, not provided), vortex to mix thoroughly, and incubate for 5 min at room temperature.**
   Unless RNase A is treated, both DNA and RNA will be co-purified. RNA can inhibit some downstream enzymatic reactions, but not PCR itself.

7. **Continue with step 5 in R. PROTOCOL FOR 3 to 5 ml of whole blood on page 43.**

Troubleshooting Guide

Facts	Possible Causes	Suggestions
Low or no recovery	**Small number of cells in the sample**	• Increase the sample volume. • Reduce the elution volume to minimum.
	Too much starting sample	• Reduce the starting sample volume or weight. • Increase the volume of lysis buffer (Buffer CL and Buffer BL) or Proteinase K solution.
	Sample is too old or incorrectly-stored	• Best result is obtained from fresh sample. DNA yield is dependent on the type, size, age, and storage condition of sample. • For blood, the amount of extracted DNA may decrease in samples stored at 4 °C for more than 5 days.
	Inefficient or insufficient lysis	• Inefficient lysis may be due to insufficient mixing with lysis buffer, too much starting sample, or degenerated Proteinase K. • Increase volume of lysis buffer in procedures, vortex the mixture vigorously and immediately to mix completely. • Proteinase K should be stored at 4 °C or below after reconstitution. For long-term stable storage, it is recommended to store at -20 °C or below.
	Improper eluent	Depending on the user's needs, other elution buffer other than Buffer AE can be used. However, the optimal elute conditions are low salt with low alkaline pH (7.0 < pH < 9.0).
Column cloggnig	**Inefficient lysis**	Check 'Inefficient or insufficient lysis' at Low or no recovery part.

Facts	Possible Causes	Suggestions
High A_{260}/A_{280}	**DNA degradation**	• Low pH or high salt in eluate can lead to high A_{260}/A_{280} result. • Use an appropriate elution buffer or repeat the washing step with Buffer TW again. • Too-old or incorrectly stored sample can obtain degraded DNA. Use fresh sample.
Low A_{260}/A_{280}	**Insufficient lysis**	Check 'Inefficient or insufficient lysis' at Low or no recovery part.
	Residual ethanol in eluate	Centrifugation at low speeds may result in ethanol remaining on the membrane, reducing purity. To completely remove ethanol, see 'Optional' centrifugation step in protocol.
	Incomplete removal of hemoglobin	Carry out additional washing step with Buffer BW before washing with Buffer TW.
DNA floats out of well while gel loading	**Residual ethanol in eluate**	Check 'Residual ethanol in eluate' at Low A_{260}/A_{280} part.
Buffer precipitation	**Buffer stored in cool ambient condition**	For proper DNA purification, the precipitate in Buffer CL and Buffer BL must be dissolved by incubation at 56 °C or higher until disappears.

Ordering Information

GeneAll® *Hybrid-Q™* *for rapid preparation of plasmid DNA*

Products	Scale	Size	Cat. No.	Type
Plasmid Rapidprep	mini	50	100-150	spin
		200	100-102	

GeneAll® *Exprep™* *for preparation of plasmid DNA*

Products	Scale	Size	Cat. No.	Type
Plasmid SV	mini	50	101-150	spin / vacuum
		200	101-102	
	Midi	26	101-226	spin / vacuum
		50	101-250	
		100	101-201	

GeneAll® *Exfection™* *for preparation of transfection-grade plasmid DNA*

Products	Scale	Size	Cat. No.	Type
Plasmid LE *(Low Endotoxin)*	mini	50	111-150	spin / vacuum
		200	111-102	
	Midi	26	111-226	spin / vacuum
		100	111-201	
Plasmid EF *(Endotoxin Free)*	Midi	20	121-220	spin
		100	121-201	

GeneAll® *Expin™* *for purification of fragment DNA*

Products	Scale	Size	Cat. No.	Type
Gel SV	mini	50	102-150	spin / vacuum
		200	102-102	
PCR SV	mini	50	103-150	spin / vacuum
		200	103-102	
CleanUp SV	mini	50	113-150	spin / vacuum
		200	113-102	
Combo GP	mini	50	112-150	spin / vacuum
		200	112-102	

GeneAll® *Exgene™* *for isolation of total DNA*

Products	Scale	Size	Cat. No.	Type
Tissue SV	mini	100	104-101	spin / vacuum
		250	104-152	
	Midi	26	104-226	spin / vacuum
		100	104-201	
	MAXI	10	104-310	spin / vacuum
		26	104-326	
Tissue Plus SV	mini	100	109-101	spin / vacuum
		250	109-152	
	Midi	26	109-226	spin / vacuum
		100	109-201	
	MAXI	10	109-310	spin / vacuum
		26	109-326	

GeneAll® *Exgene™* *for isolation of total DNA*

Products	Scale	Size	Cat. No.	Type
Blood SV	mini	100	105-101	spin / vacuum
		250	105-152	
	Midi	26	105-226	spin / vacuum
		100	105-201	
	MAXI	10	105-310	spin / vacuum
		26	105-326	
Cell SV	mini	100	106-101	spin / vacuum
		250	106-152	
	MAXI	10	106-310	spin / vacuum
		26	106-326	
Clinic SV	mini	100	108-101	spin / vacuum
		250	108-152	
	Midi	26	108-226	spin / vacuum
		100	108-201	
	MAXI	10	108-310	spin / vacuum
		26	108-326	
Genomic DNA micro		50	118-050	spin
Plant SV	mini	100	117-101	spin / vacuum
		250	117-152	
	Midi	26	117-226	spin / vacuum
		100	117-201	
	MAXI	10	117-310	spin / vacuum
		26	117-326	
Soil DNA mini	mini	50	114-150	spin
Stool DNA mini	mini	50	115-150	spin
Stool-Bead DNA mini	mini	50	115-151	spin
Viral DNA/RNA	mini	50	128-150	spin
FFPE Tissue DNA	mini	50	138-150	spin
		250	138-152	
Forensic	mini	100	122-101	spin / vacuum
		250	122-152	
cfDNA	mini	100	129-101	spin / vacuum

GeneAll® *GenEx™* *for isolation of total DNA without spin column*

Products	Scale	Size	Cat. No.	Type
GenEx™ Blood	Sx	100	220-101	solution
		500	220-105	
	Lx	100	220-301	solution
GenEx™ Cell	Sx	100	221-101	solution
		500	221-105	
	Lx	100	221-301	solution
GenEx™ Tissue	Sx	100	222-101	solution
		500	222-105	
	Lx	100	222-301	solution

Products	Scale	Size	Cat. No.	Type
GeneAll® *GenEx™* *for isolation of total DNA without spin column*				
GenEx™ Plant	Sx	100	227-101	solution
	Mx	100	227-201	
	Lx	100	227-301	
GenEx™ Plant Plus	Sx	100	228-101	solution
	Mx	50	228-250	
	Lx	20	228-320	
GeneAll® *DirEx™* series *for preperation of PCR-template without extraction*				
DirEx™		100	250-101	solution
DirEx™ *Fast*-Tissue		96 T	260-011	solution
DirEx™ *Fast*-Cultured cell		96 T	260-021	solution
DirEx™ *Fast*-Whole blood		96 T	260-031	solution
DirEx™ *Fast*-Blood stain		96 T	260-041	solution
DirEx™ *Fast*-Hair		96 T	260-051	solution
DirEx™ *Fast*-Buccal swab		96 T	260-061	solution
DirEx™ *Fast*-Cigarette		96 T	260-071	solution
GeneAll® RNA series *for preperation of total RNA*				
RiboEx™	mini	100	301-001	solution
		200	301-002	
Hybrid-R™	mini	100	305-101	spin
Hybrid-R™ Blood RNA	mini	50	315-150	spin
Hybrid-R™ miRNA	mini	50	325-150	spin
RiboEx™ LS	mini	100	302-001	solution
		200	302-002	
Riboclear™	mini	50	303-150	spin
Riboclear™ Plus	mini	50	313-150	spin
Ribospin™	mini	50	304-150	spin
Ribospin™ II	mini	50	314-150	spin
		300	314-103	
Ribospin ™ vRD	mini	50	302-150	spin
Ribospin ™ vRD Plus	mini	50	312-150	spin
Ribospin ™ vRD II	mini	50	322-150	spin
Ribospin ™ Plant	mini	50	307-150	spin
Ribospin™ Seed/Fruit	mini	50	317-150	spin
Ribospin™ Pathogen/TNA	mini	50	314-150	spin
		250	314-152	
Allspin™	mini	50	306-150	spin
RiboSaver™	mini	100	351- 001	solution

Products	Scale	Size	Cat. No.	Type
GeneAll® *AmpONE™* *for PCR amplification*				
Taq DNA polymerase		250 U	501-025	(2.5 U/μl)
		500 U	501-050	
		1,000 U	501-100	
Taq Premix		20 μl x 96 tubes	526-200	solution
		50 μl x 96 tubes	526-500	
GeneAll® *AmpMaster™* *for PCR amplification*				
Taq Master mix		0.5 ml x 2 tubes	541-010	solution
		0.5 ml x 10 tubes	541-050	solution
GeneAll® *HyperScript™* *for Reverse Transcription*				
Reverse Transcriptase		10,000 U	601-100	solution
RT Master mix		0.5 ml x 2 tubes	601-710	solution
One-step RT-PCR Master mix		0.5 ml x 2 tubes	602-110	solution
One-step RT-PCR Premix		20 μl x 96 tubes	602-102	solution
GeneAll® RealAmp™ *for qPCR amplification*				
SYBR qPCR Master mix (2X, Low ROX)	200 rxn	2 ml	801-020	solution
	500 rxn	5 ml	801-050	
SYBR qPCR Master mix (2X, High ROX)	200 rxn	2 ml	801-021	solution
	500 rxn	5 ml	801-051	
GeneAll® Protein series				
ProtinEx™ Animal cell/tissue		100 ml	701-001	solution
PAGESTA™ Reducing 5X SDS-PAGE Sample Buffer		1 ml x 10 tubes	751-001	solution

Products	Size	Cat. No.	Type
GeneAll® GENTi™ 32 ADVANCED *Newly designed automated extraction system*			
Automatic extraction equipment		GTI032A	system
Genomic DNA	48	901-048A	tube
	96	901-096A	plate
Viral DNA/RNA	48	902-048A	tube
	96	902-096A	plate
Blood DNA	48	903-048A	tube
	96	903-096A	plate
Plant DNA/RNA	48	904-048A	tube
	96	904-096A	plate
LMO	48	906-048A	tube
	96	906-096A	plate
Fecal DNA/RNA	48	913-048A	tube
	96	913-096A	plate
Forensic DNA	48	914-048A	tube
	96	914-096A	plate
Cell/Tissue Total RNA	48	915-048A	tube
	96	915-096A	plate
Plant Total RNA	48	916-048A	tube
	96	916-096A	plate
cfDNA	48	917-048A	tube
	96	917-096A	plate

Products	Size	Cat. No.	Type
Fecal DNA/RNA	48	948-048	single
	96	948-096	plate
Forensic	48	936-048	single
	96	936-096	plate
Cell/Tissue Total RNA	48	951-048	single
	96	951-096	plate
Plant Total RNA	48	952-048	single
	96	952-096	plate
cfDNA	48	953-048	single
	96	953-096	plate

GeneAll® AllEx®Mini *Compact yet Comprehensive automated extraction system*			
Automatic extraction equipment		AEX012	system
Genomic DNA	48	971-048	single
Viral DNA/RNA	48	972-048	single
Blood DNA	48	973-048	single
Plant DNA/RNA	48	974-048	single
Forensic	48	975-048	single
Fecal DNA/RNA	48	976-048	single
Cell/Tissue Total RNA	48	977-048	single
Plant Total RNA	48	978-048	single

GeneAll® AllEx®64 *Compact yet Comprehensive automated extraction system*			
Automatic extraction equipment		AEX064	system
Genomic DNA	48	931-048	single
	96	931-096	plate
Viral DNA/RNA	48	934-048	single
	96	934-096	plate
Blood DNA	48	935-048	single
	96	935-096	plate
Plant DNA/RNA	48	937-048	single
	96	937-096	plate

Used symbols and markings

REF	Catalogue number	IVD	In-vitro diagnostic medical device
LOT	Batch number	H B	Handbook code
	Expiry date		Consult instructions for use
	Manufacturer		Contains sufficient for <n> tests
	Caution		Temperature limit
	Date of manufacture	EC REP	Authorized representative in the European union
	Important note	CONC	Contains the concentrated solution. Additional material must be added before use
	Write down the current date after adding ethanol to the bottle	EtOH ? 	Mark up after adding ethanol
CE	CE-Mark		

GeneAll

GENEALL BIOTECHNOLOGY CO., LTD.
GeneAll Bldg., 303-7, Dongnamro, Songpa-gu, Seoul, 05779, Republic of Korea
E-mail: sales@geneall.com
Tel. 82-2-407-0096 Fax. 82-2-407-0779
www.geneall.com

Factory
A-1201~A-1204, Hanam Techno Valley U1 Center
947, Hanam-daero, Hanam-si, Gyeonggi-do, 12952, Republic of Korea

EC REP
HT Promedt Consulting GmbH
Ernst-Hackel-Straße 7
66386 St. Ingbert, Germany
Tel. 49 6894 581020
Fax. 49 6894 581021

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