Ver 4.3

HB1050

Handbook for

CELL SV MINI
CLINIC SV MINI
BLOOD SV MINI

exgene™

DNA PURIFICATION HANDBOOK

GeneAll

Customer & Technical Support

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

We appreciate your comments and advice.

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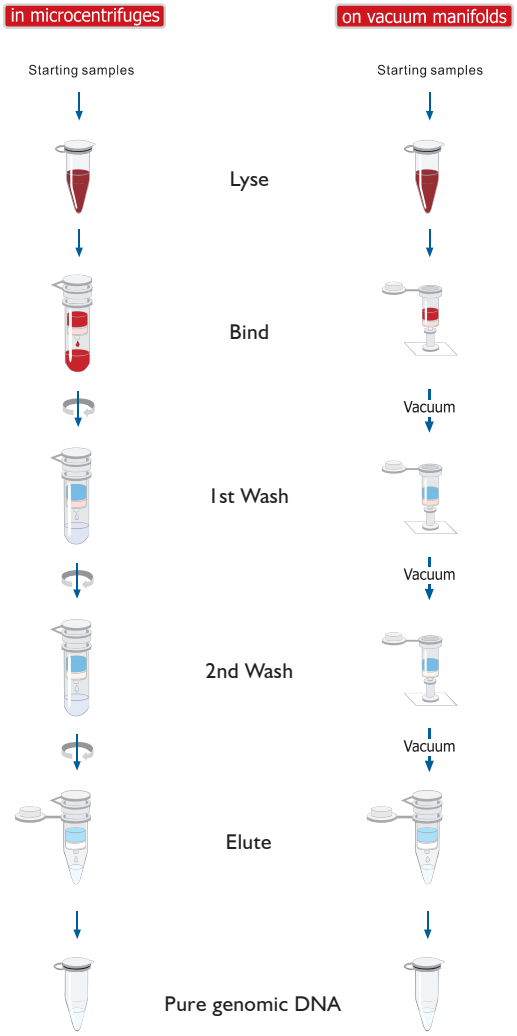
This protocol handbook is included in :

GeneAll® Exgene™ Blood SV mini (105-101, 105-152)
GeneAll® Exgene™ Clinic SV mini (108-101, 108-152)
GeneAll® Exgene™ Cell SV mini (106-101, 106-152)

Visit www.geneall.com for FAQ, Q&A and more information.

Brief 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

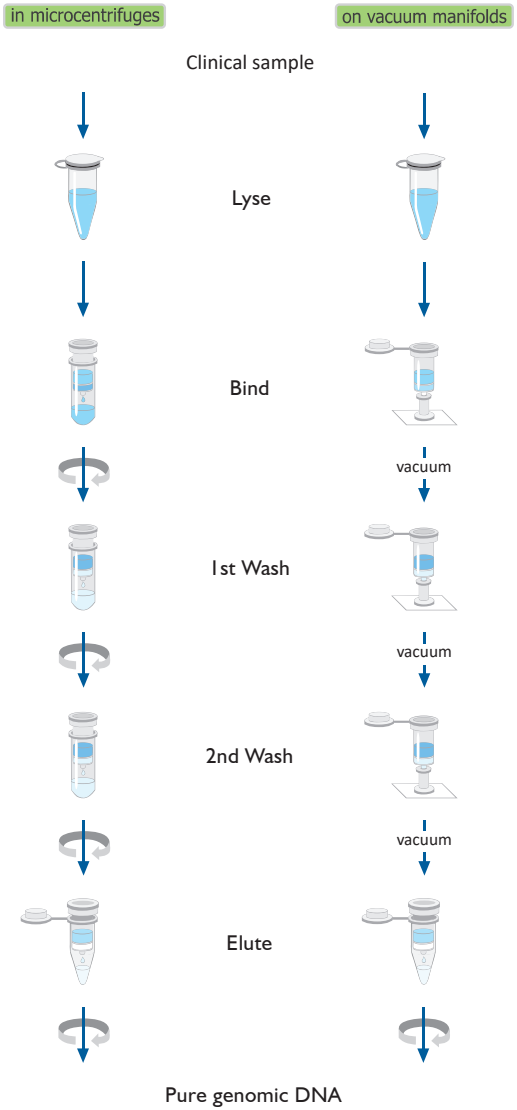
7. Transfer the mixture into mini column
8. Centrifuge for 1 min, ≥6,000 x g

9. Add 600 μl of Buffer BW into mini column
10. Centrifuge for 1 min, ≥6,000 x g

11. Add 700 μl of Buffer TW into mini column
12. centrifuge for 1 min, ≥6,000 x g
13. Additional centrifuge for 1 min ≥13,000 x g

14. Apply 200 μl Buffer AE into mini column
15. Incubate for 1 min at RT
16. Centrifuge for 1 min, ≥13,000 x g

Brief 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, ≥6,000 x g

10. Add 600 µl of Buffer BW into mini column
11. Centrifuge for 1 min, ≥6,000 x g

12. Add 700 µl of Buffer TW into mini column
13. Centrifuge for 1 min, ≥6,000 x g
14. Additional centrifuge for 1 min, ≥13,000 x g

15. Apply 200 µl Buffer AE into mini column
16. Incubate for 1 min at RT
17. Centrifuge for 1 min, ≥13,000 x g

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Kit Contents

	Blood SV		Clinic SV		Storage
Cat. No.	**105-101**	**105-152**	**108-101**	**108-152**	
Type	**mini**		**mini**		
Components	**Quantity**				
No. of preparation	100	250	100	250	Room temperature (15~25°C)
Column Type G (mini) (with collection tube)	100	250	100	250	
2 ml collection tube	200	500	200	500	
Buffer CL	-	-	25 ml	60 ml	
Buffer BL	25 ml	60 ml	25 ml	60 ml	
Buffer BW (concentrate) *	40 ml	90 ml	40 ml	90 ml	
Buffer TW (concentrate) * †	24 ml	50 ml	24 ml	50 ml	
Buffer AE **	30 ml	60 ml	30 ml	60 ml	
Proteinase K ***	48 mg	120 mg	48 mg	120 mg	
PK Storage buffer	4 ml	7 ml	4 ml	7 ml	
Protocol Handbook	1	1	1	1	

	Cell SV		Storage
Cat. No.	**106-101**	**106-152**	
Type	**mini**		
Components	**Quantity**		
No. of preparation	100	250	Room temperature (15~25°C)
Column Type G (mini) (with collection tube)	100	250	
2 ml collection tube	200	500	
Buffer GP	25 ml	60 ml	
Buffer YL	60 ml	125 ml	
Buffer CL	25 ml	60 ml	
Buffer BL	25 ml	60 ml	
Buffer BW (concentrate) *	40 ml	90 ml	
Buffer TW (concentrate) * †	24 ml	50 ml	
Buffer AE **	30 ml	60 ml	
Proteinase K ***	48 mg	120 mg	
PK Storage buffer	4 ml	7 ml	
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** Before first use, add absolute ethanol (ACS grade or better) into Buffer BW and TW as indicated on the bottle.*

† Contains sodium azide as a preservative.

*** 10 mM TrisCl, pH 9.0, 0.5 mM EDTA.*

**** For the long-term storage of Proteinase K, store at 4 °C. But after reconstitution of Proteinase K store at -20 °C. Refer to instruction of Proteinase K on page 9.*

Materials Not Provided

- Reagent : Absolute ethanol (ACS grade or better), Lysozyme, Lyticase, Zymolase
- Disposable material : RNase-free pipette tips, Sterile 1.5 ml micro-centrifuge tubes, Disposable gloves
- Equipment : Equipment for homogenizing sample, Microcentrifuge, Vortex mixer, Suitable protector

Product Specifications

Exgene™ Blood/Clinic/Cell	
Type	Spin/Vacuum
Maximum amount of starting samples	Liquid sample : 200 μl/prep Solid sample : 20 mg/prep Cultured cell : 5 x 10^6/prep
Preparation time	≥30 min
Maximum loading volume of Column Type G (mini)	750 μl
Minimum elution volume	30 μl

Quality Control

All components in Exgene™ series are manufactured in strictly clean condition, and its degree of cleanness is monitored periodically. Quality control is carried out thoroughly from lot to lot, and only the qualified kits are approved to be delivered.

Storage Conditions

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

During shipment or storage under cool ambient condition, a precipitate can be formed in Buffer BL or CL. In such a case, heat the bottle to 56°C to dissolve completely. Using precipitated buffers will lead to poor DNA recovery. Exgene™ series are guaranteed until the expiration date printed on the product box.

Safety Information

The buffers included in Exgene™ series contain irritants which are harmful when in contact with skin or eyes, or when inhaled or swallowed. Care should be taken when handling such materials. Always wear gloves and eye protection, and follow standard safety precautions.

Buffer BL 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™ cell is for research use only, not for use in diagnostic procedure.

Proteinase K

This kit provides Proteinase K and PK Storage buffer for dissolving Proteinase K. Reconstituted Proteinase K serves efficient viral lysis for most sample types.

Proteinase K solution should be stored under 4°C for conservation of activity. It can be stored at 4°C for 1 year without significant decrease in activity.

To store for extended periods of time, it is recommended to store under -20°C.

Introduction

Exgene™ series including Blood, Clinic, and Cell SV kit provide fast and easy methods for the small scale purification of total DNA from various sample species, such as blood, tissues, bacterial or cultured cells, and forensic specimens. Purified DNA can be used directly for PCR, Southern blotting, and other downstream applications.

Exgene™ series utilize the advanced silica-binding technology to purify total DNA sufficiently pure for many applications. Various samples are lysed in optimized buffer containing detergents and lytic enzyme. Under high salt condition, DNA in the lysate bind to silica membrane and impurities pass through membrane into a collection tube. The membranes are washed with a series of alcohol-containing buffer to remove any traces of proteins, cellular debris and salts. Finally pure DNA is released into a clean collection tube with deionized water or low ionic strength buffer.

This protocol can be used with:

- Exgene™ Blood SV mini (105-101/105-152)
  Fresh or frozen blood, body fluid, nucleated blood, lymphocyte, cultured cells, buccal swab, saliva, hair, sperm and etc.

- Exgene™ Clinic SV mini (108-101/108-152)
  (In addition to Blood SV's) Fresh, frozen or fixed animal tissue, dried blood spot, gram-negative bacteria and etc.

- Exgene™ Cell SV mini (106-101/106-152)
  (In addition to Clinic SV's) Gram positive bacteria, yeast and etc.

GENERAL CONSIDERATIONS

Sample amount and expected yield

Exgene™ series are designed for preparation from small amount of starting sample. Starting sample amount should not exceed the recommended maximum limit, otherwise DNA recovery will be significantly lowered (Fig. 1). Recommended amount of starting sample and the yield is listed on next page. For samples with very high DNA contents (e.g., buffy coat, spleen, which has a high cell density, and cell lines with a high degree of ploidy), less than the recommended should be used.

If your starting material is not listed or you have no information about your sample, we recommend you start with smaller sample than the listed and increase the sample size in subsequent preparation depending on the result.

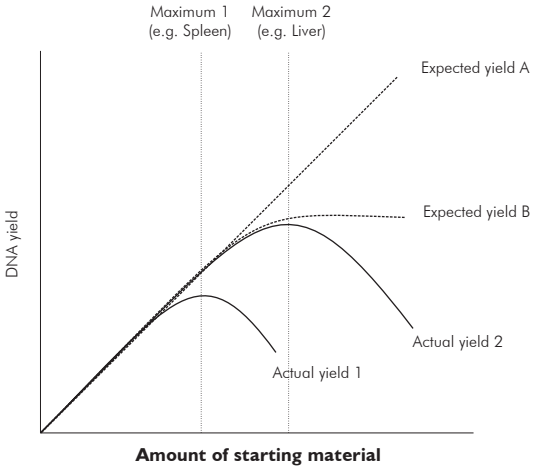


Fig. 1 The amount of starting sample should not be exceed the recommended maximum limit, otherwise DNA recovery will be significantly low. If the cell mass of starting material is high (e.g., spleen, actual yield 1), maximum capacity will be lowered (Maximum 1).

The DNA yield from whole blood will depend on the number of white blood cells (WBCs, leukocytes) contained in the sample. The number of WBCs varies from sample to sample, and can be determined using hematocytometer or other cell counter before experiment. This kit can be used to extract total DNA from blood containing as little as 2.5×10^5 leukocytes per milliliter and up to 1×10^7 cells per milliliter.

Generally, the density of bacterial cells can not be easily determined, because its optical density is influenced by various factors, such as species, media and measuring devices. Rough guide may be helpful with the bacterial cells. $A_{600}=1$ corresponds to 1×10^9 cells per milliliter with *E.coli*. For yeast, $A_{600}=1$ is obtained with a cell density of $1 \sim 2 \times 10^7$ cells per milliliter.

Sample	Starting amount (max. capacity)	Yield (μg)
Whole blood	200 μl	3~12
Buffy coat	200 μl	20~40
Nucleated blood	10 μl	5~16
Cultured cells or lymphocytes	5×10^6	14~25
Brain	20 mg	5~18
Heart	20 mg	4~10
Kidney	20 mg	15~35
Liver	20 mg	15~35
Lung	20 mg	4~10
Pancreas	20 mg	8~25
Spleen	10 mg	10~35
Bacteria	2×10^9	5~25
Yeast	5×10^7	10~25

Table 1 The yield on this table is calculated by addition of each eluate of 3 successive elution steps after DNA preparation with RNase A treatment. Without RNase A treatment, average yield from some sample may be significantly different from this data.

For preparation of DNA from larger size of starting materials than the recommended above, we recommend Exgene™ Midi or MAXI series which is capable of processing the larger samples; On average, 4 times (Midi) and 10 times (MAXI) to mini series (See ordering information at page 45).

Sample preparation

The yield and purity of DNA can be varied depending on the methods for harvesting and/or storing the starting sample materials. Freshly harvested sample should be used or stored immediately for best result. Note that the sample should be handled on ice as quickly as possible and repeated freezing and thawing of frozen sample should be avoided.

Blood and its derivatives

Blood sample should be used or stored immediately after collected to the tubes containing the anticoagulants and the preservatives for whole blood. Whole blood collected in anticoagulants, such as EDTA or citrates (CPDs and ACDs), can be stored for several days at 4°C and at least for 2 years at -80°C without significant change in its properties. EDTA, a metal chelator, is an inhibitor against metal-dependent nuclease and is most preferable anticoagulant for DNA preparation. Heparin can also be used as anticoagulant but is not usually used because it acts as an inhibitor in PCR reaction. Frozen blood should be thawed quickly in 37°C water bath and kept on ice before use.

The derivatives, such as plasma, serum or buffy coat, can also be used for specific application. Buffy coat can be used for higher yield of DNA and is prepared by collecting the intermediate phase after the centrifugation of whole blood.

150~250 μl of buffy coat can be collected from 3 ml of whole blood, but the concentration of leukocytes should be determined because overload of leukocyte will lead to poor result. If the number of leukocytes exceeds 5×10^6, DNA recovery will be significantly decreased.

Cultured cells

Cultured cells growing in suspension can be easily harvested by centrifugation. However attached cells should be treated with trypsin-EDTA for detaching the cells before harvesting. The number of cells should be determined using a hematocytometer or other cell counter, and should not be over 5×10^6 per prep. Harvested cells washed with phosphate buffered saline (PBS) can be used directly in DNA preparation or stored at -20°C or -80°C in pellet. It is not recommended washing the fixed cells with PBS, because it can cause cell lysis and significant reduce in DNA yield. Sample should always be kept on ice before use.

Tissue

Harvested tissues should be used freshly or stored at very low temperature as quickly as possible. Generally, homogenizing the sample finely will lead to not only better result, but also reduction of experiment-time. Grinding in mortar and pestle under liquid nitrogen is a good method for disrupting the sample, but alternative methods, such as a homogenizer or a bead-beater, can be employed in case by case for efficient disruption. Shaking or vortexing during incubation for lysis may greatly accelerate the efficiency of lysis, resulting in reduced time for complete lysis. Note that the freshness and the particle size of disrupted sample is the key for good result and that the frozen sample should be kept on ice until use.

Bacterial cells

Bacterial cells can be prepared by incubating the culture for 12~24 hours at 37°C with vigorous shaking until the cell reach the log phase. Harvested bacterial cells can be used directly or stored at -20°C or -80°C for future use. Gram positive bacteria should be treated with lysozyme or lysostaphin to weaken the rigid and multilayered cell wall, while gram negative bacteria does not need to. Extreme care should be taken for pathogenic bacteria.

Yeast cells

Yeast cells are troublesome for purification of DNA, because its rigid cell wall does not lysed well in usual lytic condition. The cell wall of yeast should be loosened by enzymatic lysis with an lytic enzyme such as lyticase or zymolase, and spheroplasts are then collected by centrifugation. These harvested spheroplasts can be used directly for DNA preparations or stored at -20°C or -80°C for later use. When the value of A_{600} is 1, the cell density of yeast culture may be $1 \sim 2 \times 10^7$ cells per milliliter.

Elution

Purified DNA is eluted from Column Type G (mini) in either sterilized water or Buffer AE which contains 0.5 mM EDTA and 10 mM Tris-HCl, pH 9.0. Elution buffer should be equilibrated to room temperature before applying to mini column.

Typically, elution is carried out in two successive steps using 200 μl Buffer AE each time. The volume of elution can be adjusted depending on the starting materials or the downstream applications, but it should be over the minimum requirements to wet the entire column membrane (50 μl per column) and should not be over 300 μl.

Basically, it is recommended for the recovery of higher DNA concentration to decrease the elution volume to minimum, but total DNA recovery will decrease in this case. Otherwise, if maximum recovery is needed, the volume of elution buffer should be increased to elute as much as possible. Yield may be slightly increased if the mini column is incubated with the elution buffer at room temperature for 5 min before centrifugation.

Generally, DNA bound to the mini column will not be eluted completely with a single elution step. Approximately 60~85% of the DNA will elute in the first 200 μl, and the rest of bound DNA in next 200 μl (Fig. 2). However, a single elution with 200 μl of elution buffer will be sufficient to recover the amount of DNA required for multiple PCR reactions. For very small samples (containing less than 1 μg of DNA), only a single elution in 50 μl of Buffer AE or sterilized water is recommended.

The mini column for Exgene™ series co-purifies DNA and RNA when both are present in the sample. RNA may inhibit some downstream enzymatic reactions, although it does not inhibit PCR itself. If RNA-free DNA is required, RNase A should be treated at the optional step included in each protocol. A treatment of RNase A will decrease the overall yield measured by spectrophotometer, but the virtual recovery of DNA will be slightly increased. RNase A can be purchased at GeneAll Biotechnology (www.geneall.com), but any equivalent can be used at the concentration of 20 mg/ml.

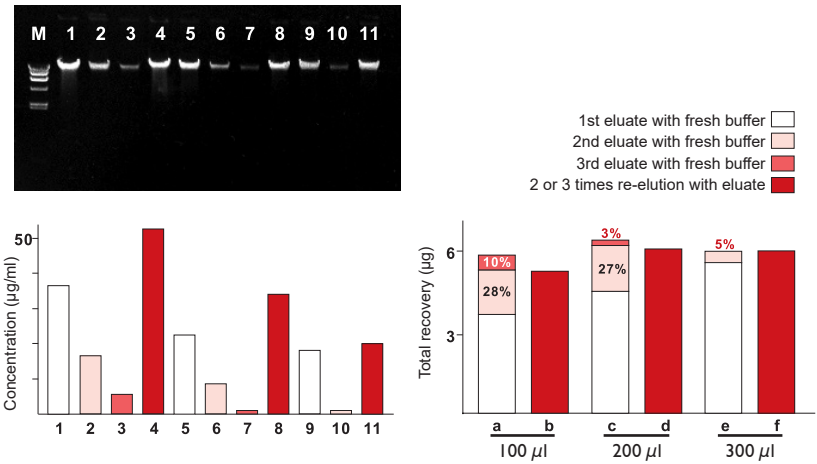
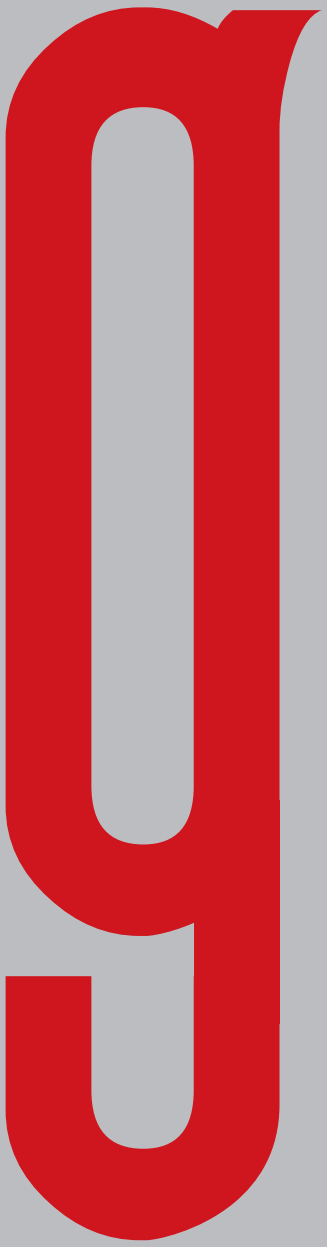


Fig. 2 The yield and concentration of purified DNA depending on the elution method.
DNA was prepared from 200 μl of bovine whole blood. Each preparation was exactly identical except the elution method; Elution was performed 3 times per column with 100 μl (lane 1~3) and 200 μl (lane 5~7), and 2 times per column with 300 μl (lane 9~10) of fresh Buffer AE. At the same time, another elution was carried out 3 times (100 μl and 200 μl) and 2 times (300 μl) by recursive use of the eluate instead of fresh Buffer AE (lane 4, 8, 11). Total 11 eluates purified from 6 samples were resolved on 0.8% agarose gel to visualize (upper left) and its concentration (lower left) and total yield (lower right) was measured by spectrophotometric analysis.

GeneAll® Exgene™ PROTOCOLS

Read the protocol carefully before experiment.



A. PROTOCOL FOR BLOOD AND 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 the water bath to 56°C.*
- *Prepare absolute ethanol.*
- *Prepare 1.5 ml microcentrifuge tube.*
- *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.*

This protocol can be used with Blood/Clinic/Cell SV mini

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 1X 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 1X PBS
Cultured cells or lymphocyte	5 x 10^6 cells	5 x 10^6 cells in 200 µl of 1X 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 2~3 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.
   Longer incubation will not affect DNA recovery.

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 6,000 x g above (>8,000 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 (>13,000 x g) 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 6,000 x g above (>8,000 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 6,000 x g above (>8,000 rpm) for 1 min. Discard the pass-through and reinsert 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 (13,000~20,000 x g).

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

** For low cell-density sample, such as body fluids or virus, use 50~150 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 starting sample amount is very small, second elution can be carried out with first eluate instead of fresh elution buffer. Alternatively for higher concentration, elution volume can be decreased to 50 μl. However the small volume of elution buffer will decrease the total yield of DNA recovery.

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 the water bath to 56°C.*
- *Prepare sterile sharp blade (or wire cutter) and tweezer.*
- *Prepare 1X PBS and absolute ethanol.*
- *Prepare 1.5 ml microcentrifuge tube and 2.0 ml microcentrifuge tube.*
- *Equilibrate Buffer AE to room temperature.*
- *All centrifugation should be performed at room temperature.*
- *If precipitate has formed in Buffer BL, heat to dissolve at 56°C.*

This protocol can be used with Blood/Clinic/Cell SV mini

* Due to the need of additional Buffer BL for buccal swab, fewer preparations can be performed. Buffer BL can be purchased separately as accessory (105-901).

1. **Scrape the swab firmly more than 5~6 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 1X 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 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 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. Briefly centrifuge to remove any drops from inside the lid.**

6. **Add 400 µl of absolute ethanol (not provided) to the lysate, and mix well by vortexing. Briefly centrifuge 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 6,000 x g above (>8,000 rpm) for 1 min. Discard the pass-through and reinsert the mini column back into the collection tube.**
   Be careful not to wet the rim of the mini column.

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 BLOOD AND BODY FLUID/CULTURED CELLS USING MICROCENTRIFUGE on page 20.**

C. PROTOCOL FOR SALIVA AND MOUTHWASH

Before experiment

- *Before first use, add absolute ethanol (ACS grade or better) into Buffer BW and TW as indicated on the bottle.*
- *Prepare the water bath to 56°C.*
- *Prepare 1.5 ml microcentrifuge tube and 50 ml conical tube.*
- *Prepare 1X PBS (Phosphate buffered saline) and absolute ethanol.*
- *Equilibrate Buffer AE to room temperature.*
- *All centrifugation should be performed at room temperature.*
- *If precipitate has formed in Buffer BL, heat to dissolve at 56°C.*

This protocol can be used with Blood/Clinic/Cell SV mini

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 1X 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 2,000 x g (3,000 rpm) for 5 min to pellet cells. Immediately and carefully decant the supernatant to prevent loose cell pellets. Resuspend completely the pellets in 200 µl of 1X 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 copurified 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.**
   For efficient lysis, mix the sample completely.

5. **Continue with step 4 in A. PROTOCOL FOR BLOOD AND BODY FLUID/CULTURED CELLS USING MICROCENTRIFUGE on page 19.**

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 the 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 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 0.5~1 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. Spin down 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 BLOOD AND BODY FLUID/CULTURED CELLS USING MICROCENTRIFUGE on page 19.**

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 the 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 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. Spin down 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 BLOOD AND BODY FLUID/CULTURED CELLS USING MICROCENTRIFUGE on page 19.**

F.

PROTOCOL FOR
BLOOD AND 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 the water bath to 56 °C.*
- *Prepare absolute ethanol.*
- *Prepare 1.5 ml microcentrifuge tube.*
- *Prepare vacuum system; manifold, trap, tubing and pump capable of 15~20 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.*

This protocol can be used with **Blood/Clinic/Cell SV mini**

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 1X 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 1X PBS
Cultured cells or lymphocyte	5 x 10^6	5 x 10^6 cells in 200 μl of 1X 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 2~3 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.
Longer incubation will not affect DNA recovery.

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 19).

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 19.

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 <u>A. PROTOCOL FOR BLOOD AND BODY FLUID/CULTURED CELLS USING MICROCENTRIFUGE</u> on page 20.**

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 the water baths or incubators to 56 °C and 70 °C.*
- *Prepare absolute ethanol.*
- *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 microhomogenizer 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 sec.

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 sec.

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

This protocol can be used with Clinic/Cell SV mini

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 (2~3 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 copurified 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 6,000 x g above (>8,000 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 (>13,000 x g) 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 6,000 x g above (>8,000 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 (>13,000 x g) will not affect DNA recovery.

8. **Apply 700 µl of Buffer TW. Centrifuge at 6,000 x g above (>8,000 rpm) for 1 min. Discard the pass-through and reinsert the mini column back into the collection tube.**
   Centrifugation at full speed will not affect DNA recovery.

9. **Centrifuge at full speed (>13,000 x g) 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 (13,000~20,000 x g).

10. Add 200 μl of Buffer AE or sterilized water. Incubate at room temperature for 1 min. Centrifuge at full speed (>13,000 x g) 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 50~150 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 xylene and absolute ethanol.*
  Xylene is an irritant and appropriate precautions should be taken in handling. For example, wear gloves, safety goggles, and a laboratory coat, avoid contact with skin, eyes and clothing and work in a fume hood.
- *Prepare the water bath to 56 °C and 70 °C.*
- *Prepare 2.0 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. **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 1,200 µl xylene. Vortex vigorously until the paraffin has been completely melted. Centrifuge at full speed (>13,000 x g) for 5 min. Carefully remove supernatant by pipetting.**
   Be careful not to lose any of the pellet.

3. **Add 1,200 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 steps 3~4 once or twice.**

6. **Evaporate the residual ethanol by incubating the microcentrifuge tube at room temperature for 10~15 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 31.**

This protocol can be used with Clinic/Cell SV mini

I.

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.*
- *Prepare the 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. **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 1X 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 31.**

This protocol can be used with Clinic/Cell SV mini

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 water baths or incubators 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 CL and BL 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 3~4 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. Spin down 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 31.**

This protocol can be used with Clinic/Cell SV mini

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 water baths or incubators to 56°C and 70°C.*
- *Prepare 1.5 ml microcentrifuge tube.*
- *Prepare absolute ethanol.*
- *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.**
   1~2 ml of overnight bacterial culture (A_{600}=1) may correspond to 1~2 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 31.**

This protocol can be used with Clinic/Cell SV mini

L.

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 water baths or heating blocks to 37°C, 56°C and 70°C.*
- *Prepare Lysozyme (LYS702, Bioshop, Canada, or equivalent) or Lysostaphin (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 CL and BL 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 the supernatant.**

2. **Resuspend the cell pellet thoroughly in 180 μl of the prepared enzyme mixture. Incubate at 37°C for 30 min.**
   The purpose of this treatment is to weaken the cell wall so that efficient cell lysis can take place.

This protocol can be used with Cell SV mini

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 <u>G. PROTOCOL FOR ANIMAL TISSUE</u> on page 31.**

M. PROTOCOL FOR YEAST

Before experiment

- *Before first use, add absolute ethanol (ACS grade or better) into Buffer BW and TW as indicated on the bottle.*
- *Prepare water baths or incubators to 37°C, 56°C, and 70°C.*
- *Prepare the enzyme for lysing the cell wall; lyticase or zymolase.*
- *Prepare 1.5 ml microcentrifuge tube or 2.0 ml microcentrifuge tube or 15 ml conical tube.*
- *Prepare absolute ethanol.*
- *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. **Add 3 ml (up to 5 x 10^7 cells) of a culture grown in YPD broth to a 15 ml conical tube. Centrifuge at 5,000 x g for 10 min to pellet the cells. Discard the supernatant.**
   Alternatively, harvest twice in 1.5 ml or 2.0 ml microcentrifuge tube. If 1.5 ml or 2.0 ml microcentrifuge tube is employed, centrifuge at full speed (>13,000 x g) for 1 min, discard the supernatant and repeat again with the remainder.
   When the value of A_{600} reaches to 1.0 (generally, log-phase), 3 ml of culture may yield approximately 10~25 μg of DNA .

2. **Resuspend the pellet thoroughly in 500 μl of Buffer YL.**

3. **Add 200 U of lyticase (not provided) or 20 U of zymolase (not provided) and gently pipet to mix completely.**
   Unit/mg of lyticase varies depending on the manufactures.

4. **Incubate at 37°C for 30~60 min to digest the cell wall.**
   Incubated cells turn to spheroplasts at this step, and this makes it easy the followed lysis step.

5. **Centrifuge at 5,000 x g for 5 min. Discard the supernatant.**
   If 1.5 ml or 2.0 ml microcentrifuge tube is used, centrifuge at full speed for 1 min.

This protocol can be used with Cell SV mini

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

7. **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.

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

9. **Continue with step 3 in G. PROTOCOL FOR ANIMAL TISSUE on page 31.**

Troubleshooting Guide

Facts	Possible Causes	Suggestions
Low or no recovery	**Low cells in the starting sample**	Some samples may have low concentration of cells, and some whole blood may contain low concentration of white blood cells. Increase the sample volumes and load the mini column several times. Reduce the elution volume to minimum. When the cell mass is low, it is also recommended to use carrier RNA (e.g. Poly-dN, glycogen, or tRNA dissolved at 20~40 μg/ml in Buffer BL).
	Too many cells in the starting sample	Sample amount over the maximum capacity will lead to poor lysis, resulting in significantly low recovery. Reduce the amount of starting sample or increase the volume of buffers proportionally.
	Inefficient or insufficient lysis	Inefficient lysis may be due to several causes; - Insufficient mixing with Buffer BL - Too much cells in the starting sample - Degenerated Proteinase K - Poor disruption of tissue After addition of Buffer BL in protocol, vortex the mixture vigorously and immediately to mix completely. If too much cells present in the sample, reduce the starting sample volume, or increase the volume of Buffer BL to double. Using tissue as sample material, lysis should be continued until the tissue is completely lysed. Completely lysed sample will not have any particulate in lysate. Proteinase K should be stored under 4°C for maintenance of proper activity. However, it is recommended to store in small aliquots at -20°C for prolonged preservation of its activity.
	Improper eluent	As user's need, elution buffer other than Buffer AE can be used. However, the condition of optimal elution should be low salt concentration with alkaline pH (7.0<pH<9.0). When water or other buffer was used as eluent, ensure that condition.

Facts	Possible Causes	Suggestions
mini column has colored residue associated with it after wash, resulting in colored residue	**Insufficient lysis**	Insufficient lysis may cause that colored residue remains on the mini column membrane. Repeat the procedure after consideration of 'Inefficient or insufficient lysis' at "Low or no recovery".
	Incomplete removal of hemoglobin	In case of DNA preparation from certain animal blood, it is hard to remove hemoglobin from their blood. Carry out additional wash step with Buffer BW before washing with Buffer TW.
Column clogging	**Inefficient lysis**	Inefficient lysis may lead to column clogging. About inefficient lysis, check 'Inefficient or insufficient lysis' at "Low or no recovery"
High A_{260}/A_{280} ratio	**RNA contamination**	RNA may inhibit some downstream enzymatic reactions. If RNA-free DNA is required, carry out RNase A treatment on protocol optionally.
Low A_{260}/A_{280} ratio	**Insufficient lysis**	Insufficient lysis cause low DNA purity, and is due to insufficient mixing with Buffer BL, too much cells in the starting sample, or degenerated Proteinase K. Check these out on next preparations.
	Incomplete removal of hemoglobin	In case of DNA preparation from certain animal blood, it is hard to remove hemoglobin from their blood. Carry out additional wash step with Buffer BW before washing with Buffer TW.
Low concentration of DNA in eluate	**Low cells in starting sample (too high elution volume)**	Increase the volume of starting sample with additional volume of buffer used, and/or reduce the elution volume to 50 μl or do re-elution with eluate. For higher concentration of DNA in eluate, refer to the 'Elution' section of General considerations at page 15.

Facts	Possible Causes	Suggestions
Degraded DNA	**Starting sample is too old or mis-stored**	Too old or mis-stored sample often yield degraded DNA. Use fresh sample.
DNA floats out of well while loading of agarose gel	**Residual ethanol from Buffer TW remains in eluate**	Ensure that Buffer TW wash step in protocol has been performed correctly. Mini column membrane should be completely dried via additional centrifugation or air-drying. Refer the annotation of Buffer TW washing step.
Enzymatic reaction is not performed well with purified DNA	**Low purity of DNA**	Check "Low A_{260}/A_{280} ratio"
	RNA contamination	RNA may inhibit some downstream enzymatic reactions. If RNA-free DNA is required, carry out RNase treatment step optionally.
	Incomplete removal of hemoglobin	In case of DNA preparation from certain animal blood, it is hard to remove hemoglobin from their blood. Carry out additional wash step with Buffer BW before washing with Buffer TW.
	High salt concentration in eluate	Ensure that all washing steps were performed just in accordance with the protocols. Alternatively, carry out additional washing step with Buffer TW. It may help remove high salt in eluate.
Precipitate in Buffer BL or CL	**Buffer stored in cool ambient condition**	For proper DNA purification, any precipitate in Buffer BL/CL should be dissolved by incubating the buffer at 56°C or above until it 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	

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
GeneAll® AllEx®64 *Compact yet Comprehensive automated extraction system*			
Automatic extraction equipment		AEX064	system
Genomic DNA	48	931-048A	tube
	96	931-096A	plate
Viral DNA/RNA	48	934-048A	tube
	96	934-096A	plate
Blood DNA	48	935-048A	tube
	96	935-096A	plate
Plant DNA/RNA	48	937-048A	tube
	96	937-096A	plate
Fecal DNA/RNA	48	948-048A	tube
	96	948-096A	plate

GeneAll

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