

Handbook for

- *GenEx*<sup>TM</sup> Blood
- *GenEx*<sup>TM</sup> Cell
- *GenEx*<sup>TM</sup> Tissue

TOTAL DNA PURIFICATION KIT

*genex*<sup>tm</sup>

## 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](http://www.geneall.com)

Tel : 82-2-407-0096

Fax : 82-2-407-0779

E-mail (Order/Sales) : [sales@geneall.com](mailto:sales@geneall.com)

E-mail (Tech. Info.) : [tech@geneall.com](mailto:tech@geneall.com)

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This protocol handbook is included in :

GeneAll® GenEx™ Blood (220-101, 220-105, 220-301)

GeneAll® GenEx™ Cell (221-101, 221-105, 221-301)

GeneAll® GenEx™ Tissue (222-101, 222-105, 222-301)

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# Kit Contents



## For Blood / GenEx™ Blood

Cat. No.	220-101	220-105	220-301
Size	Sx *	Sx *	Lx **
No. of preparation	100 *	500 *	100 **
Buffer RL (RBC Lysis Solution)	100 ml	500 ml	3.3 L
Buffer AL (Cell Lysis Solution)	35 ml	165 ml	1.1 L
Buffer PP (Protein Precipitation Solution)	12 ml	60 ml	350 ml
Buffer RE *** (DNA Rehydration Solution)	12 ml	60 ml	90 ml
Protocol Handbook	1	1	1

## For Cultured Cell / GenEx™ Cell

Cat. No.	221-101	221-105	221-301
Size	Sx *	Sx *	Lx **
No. of preparation	100 *	500 *	100 **
Buffer AL (Cell Lysis Solution)	35 ml	165 ml	1.6 L
Buffer PP (Protein Precipitation Solution)	12 ml	60 ml	550 ml
Buffer RE *** (DNA Rehydration Solution)	6 ml	30 ml	110 ml
RNase Solution (20 mg/ml)	120 $\mu$ l	600 $\mu$ l	3 ml
Protocol Handbook	1	1	1

## For Tissue / GenEx™ Tissue

Cat. No.	222-101	222-105	222-301
Size	Sx *	Sx *	Lx **
No. of preparation	100 *	500 *	100 **
Buffer AL (Cell Lysis Solution)	35 ml	165 ml	330 ml
Buffer PP (Protein Precipitation Solution)	12 ml	60 ml	110 ml
Buffer RE *** (DNA Rehydration Solution)	6 ml	30 ml	110 ml
Proteinase K	5 mg	20 mg	33 mg
PK Storage Buffer	2 ml	2 ml	2 ml
RNase Solution (20 mg/ml)	120 $\mu$ l	600 $\mu$ l	600 $\mu$ l
Protocol Handbook	1	1	1

\* On the basis of DNA purification from 300  $\mu$ l whole blood,  $2 \times 10^6$  cells or 10 mg animal tissue

\*\* On the basis of DNA purification from 10 ml whole blood,  $1 \times 10^9$  cells or 100 mg animal tissue

\*\*\* 10 mM TrisCl, pH 8.0, 1 mM EDTA



## Product Disclaimer

GeneAll® *GenEx*<sup>TM</sup> kits are for research use only, and should not be used for drug, household or other unintended uses. All due care and attention should be taken in every procedure in this handbook. Please consult the Material Safety Data Sheet (MSDS) for information regarding hazards and safe handling practices.

## Storage and Stability

GeneAll® *GenEx*<sup>TM</sup> kits are shipped at room temperature. Basically all components in these kits are stable at room temperature (15~25°C). But for enzymes, RNase A and Proteinase K, it is recommended to store under 4°C for prolonged activity. At first use, Proteinase K should be reconstituted using PK Storage Buffer and it can be stored under 4°C until the expiration date without a significant decrease in its activity.

A precipitate can be formed in Buffer AL under cool ambient condition. In such a case, heat the bottle at 56°C until completely dissolving.

## Safety Information

Buffer AL and PP contain irritant which is harmful when in contact with skin or eyes, or when inhaled or swallowed. Care should be taken during handling. Always wear gloves and eye protector, and follow standard safety precautions.

## Quality Control

All components in GeneAll® *GenEx*<sup>TM</sup> kits are manufactured in strictly clean condition, and its degree of cleanness is monitored periodically. Restriction enzyme assay, PCR amplification assay and spectrophotometric assay as the validation of quality are carried out from lot to lot thoroughly, and only the qualified is approved to deliver.

# DNA Yields from Various Starting Materials

Materials	Species	Amount	Yields of DNA
Whole blood *	Human	300 $\mu$ l	5~15 $\mu$ g
		3 ml	80~150 $\mu$ g
		10 ml	250~500 $\mu$ g
	Mouse	300 $\mu$ l	6~7 $\mu$ g
Buffy coat *	Human	150~250 $\mu$ l	50~150 $\mu$ g
Body fluids	Human	50 $\mu$ l	0.1~2.5 $\mu$ g
Cultured cell lines	CHO	2 x 10 <sup>6</sup> cells	14~16 $\mu$ g
	RAW264.7	2 x 10 <sup>6</sup> cells	16~17 $\mu$ g
	COS	1.5 x 10 <sup>6</sup> cells	9~12 $\mu$ g
	K562	3 x 10 <sup>6</sup> cells	15~30 $\mu$ g
	NIH3T3	2 x 10 <sup>6</sup> cells	9~13 $\mu$ g
	PC12	8 x 10 <sup>6</sup> cells	5~8 $\mu$ g
Animal tissue	Mouse Liver	10 mg	20~25 $\mu$ g
	Mouse Pancreas	10 mg	70~75 $\mu$ g
	Mouse Heart	10 mg	2~4 $\mu$ g
	Mouse Tail	1 cm of tail tip	15~30 $\mu$ g
Gram (-) bacteria	E.Coli/JM109	2 x 10 <sup>9</sup> cells	18~25 $\mu$ g
	E.cloacae	6 x 10 <sup>9</sup> cells	20~26 $\mu$ g

\* Yield depends on the quantity of white blood cells present

## Introduction

GenEx™ Series provide convenient methods for the isolation of total DNA from various biological samples without use of toxic chemical such as phenol or chloroform. These kits utilize the specially formulated buffer system in order to process the sample scalably and obtain the almost intact size of genomic DNA. Extracted genomic DNA can be applied directly to PCR, Southern blotting and restriction enzyme assay and other downstream applications.

**GenEx™ Series can be used for;**

GenEx™ Blood - Whole blood and blood derivatives

GenEx™ Cell - Cultured cells and gram negative bacteria

GenEx™ Tissue - Animal tissues

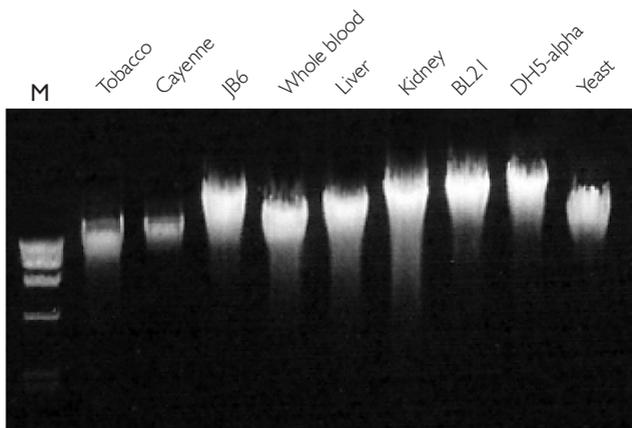
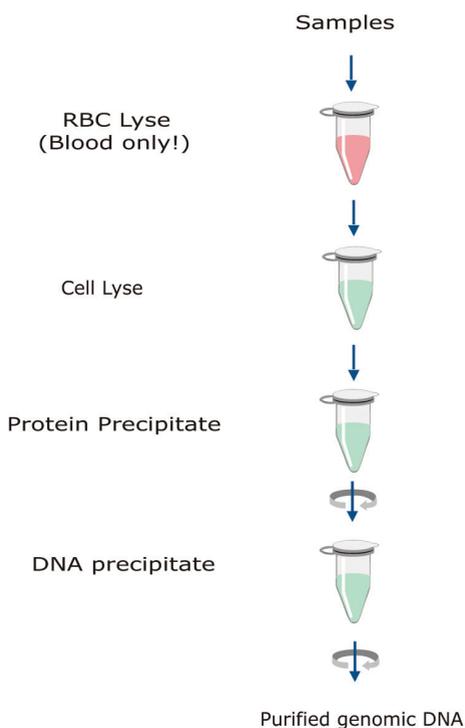


Fig. 1 Genomic DNA prepared from several kinds of organism using GenEx™ Genomic DNA purification kit. 5  $\mu$ l of eluate from each sample was resolved on 0.7% agarose gel.

# GenEx™ Kits Procedures

DNA Purification procedures of GenEx™ kits consist of four-step processes. The first step in this procedure is the lysis of cells and nuclei. RNA digestion step may be included at this time depending on each application. The cellular proteins are removed by addition of Protein Precipitation Buffer (Buffer PP), which precipitate protein but leaves the DNA in the supernatant. Finally DNA is concentrated and desalted by isopropanol precipitation.



## General Considerations



### Sample preparation

The yield and purity of DNA depend on the methods for harvesting and/or storing the starting sample materials. For best result, fresh sample should be used or stored immediately after harvesting. Note that the sample should be handled as quickly as possible and repeated freezing and thawing of frozen sample should be avoided. Considerations for harvest and storage of various sample materials are discussed below.

### Blood

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 it is not usually used as anticoagulant because it acts as an inhibitor in PCR reactions. Frozen blood should be thawed quickly in 37°C water bath and kept on ice before use. The fresher blood sample generally yields better result in DNA preparation. The derivatives, such as plasma, serum or buffy coat, can also be used for specific application.

## Cultured cells

Cells growing 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 hemacytometer or other cell counter. Harvested cells washed with phosphate buffered saline (PBS) can be used directly in DNA preparation or stored at  $-20^{\circ}\text{C}$  or  $-80^{\circ}\text{C}$  in pellet. It is not recommended washing fixed cells with PBS, because it can cause cell lysis and significant reduce in DNA yield. Before use, sample should always be kept on ice.

## Tissues

Harvested tissues (animal) should be used freshly or stored at very low temperature as quickly as possible. To make the sample finer will result better yield and quality of DNA. Generally, grinding in mortar and pestle under liquid nitrogen is a good method for disrupting the sample. Shaking or vortexing during incubation for lysis may greatly accelerate the efficiency of lysis. Alternatively, tissue samples can be effectively disrupted using some instruments, such as a rotor-stator homogenizer or a bead-beater.

Note that the freshness and the particle size of ground sample is the key for good result and that the sample should be kept on ice until use.

## Bacteria

Incubate the culture for 12~18 hours at  $37^{\circ}\text{C}$  with vigorous shaking until the cell reach the log phase. Harvest the bacterial cells from the culture by centrifugation. Decant the supernatant carefully and then use immediately or store the cells at  $-20^{\circ}\text{C}$  or  $-80^{\circ}\text{C}$ .

## Protein precipitation

Many unwanted components included in cell lysate, such as RNAs, carbohydrates and proteins (the majority) can be removed by several methods such as precipitation. There are some methods for precipitating the proteins by decreasing the solubility; At low concentration of salts the solubility of proteins usually increase slightly, but at high concentration of salts the solubility of proteins drops sharply. Changing the pH of the mixtures is an alternative for precipitating the proteins and this effect is due to the different functional groups on a protein.

The addition of Buffer PP to the lysate will induce the precipitation of proteins and detergents by the combined effect, without use of harmful organic solvent.

## DNA precipitation

Alcohol precipitation is a usual method to concentrate nucleic acid, and it can be achieved by addition of 2 volumes of ethanol or 0.6 volumes of isopropanol in the presence of mono cation.

Alcohol removes hydration shell (capsid) of DNA and then uncovers phosphate group which has negative charge. Uncovered phosphate group is neutralized by positive ion, such as  $\text{Na}^+$ , followed by precipitation of DNA due to the loss of solubility to water.

When the cell number of starting sample is very low, the consequent yield will be also very low. It is because the precipitation of DNA can not be taken place properly when small concentration of DNA. In this case, some nucleic acid carrier, such as tRNA or glycogen, should be added before addition of ethanol or isopropanol. Precipitated DNA is washed by 70% ethanol and air-dried before re-hydration with water.

# Quantities of Buffer for Various Sample Amounts and DNA Yield.

GenEx™ kits provide information about quantities of buffer for use with various sample amounts. The obtained DNA yield will depend on the storage condition, the sample type, and the number of cells in starting sample.

**Table 1.** Buffer volumes for scaling of whole blood protocols

Whole blood (ml)	0.1	0.3	0.6	1	3	5	10
Tube size (ml)	1.5	1.5	15	15	15	50	50
Buffer RL (ml)	0.3	0.9	1.8	3	9	15	30
Buffer AL (ml)	0.1	0.3	0.6	1	3	5	10
RNase A (μl)	1	1	1.5	2	6	10	20
Buffer PP (ml)	0.05	0.1	0.2	0.33	1	1.6	3.3
Isopropanol (ml)	0.1	0.3	0.6	1	3	5	10
70% EtOH (ml)	0.1	0.3	0.6	1	3	5	10
Buffer RE (μl) *	30	100	150	200	250	500	800
DNA yield (μg) **	1~5	3~15	6~30	10~50	30~150	50~250	100~500

\* The volume of Buffer RE can be adjusted depending on the target concentration.

\*\* The fresher blood sample generally yields higher DNA yield.

Low concentration of WBCs may lead to poor yield.

**Table 2.** Buffer volumes for scaling of cultured cell protocols

Cell number	$5.0 \times 10^5$	$1.0 \times 10^6$	$2.0 \times 10^6$	$1.0 \times 10^7$	$2.0 \times 10^7$	$6.0 \times 10^7$	$1.0 \times 10^8$
Tube size (ml)	1.5	1.5	1.5	2	15	50	50
Buffer AL (ml)	0.075	0.15	0.3	1.5	3	10	15
RNase A ( $\mu$ l)	1	1	1	3	6	20	30
Buffer PP (ml)	0.03	0.05	0.1	0.5	1	3.3	5
Isopropanol (ml)	0.075	0.15	0.3	1.5	3	10	15
70% EtOH (ml)	0.075	0.15	0.3	1.5	3	10	15
Buffer RE ( $\mu$ l) *	25	25	50	150	300	800	1000
DNA yield ( $\mu$ g) **	2~4	5~8	10~16	50~80	100~160	300~480	500~800

\* The volume of Buffer RE can be adjusted depending on the target concentration.

\*\* The yield of DNA will vary considerably depending on the cell number.

**Table 3.** Buffer volumes for scaling of tissue protocols

Weight of tissue (mg)	5	10	50	100
Tube size (ml)	1.5	1.5	15	15
Buffer AL (ml)	0.15	0.3	1.5	3
Proteinase K ( $\mu$ l)	1	1.5	7.5	15
RNase A ( $\mu$ l)	1	1	3	6
Buffer PP (ml)	0.05	0.1	0.5	1
Isopropanol (ml)	0.15	0.3	1.5	3
70% EtOH (ml)	0.15	0.3	1.5	3
Buffer RE ( $\mu$ l) *	50	100	350	600
DNA yield ( $\mu$ g) **	10~12	20~24	100~120	200~240

\* The volume of Buffer RE can be adjusted depending on the target concentration.

\*\* The yield of DNA will vary considerably depending on the tissue type.

**A****PROTOCOL**  
**for 300  $\mu$ l of Whole Blood****[ GenEx™ Blood kit ]**

Before proceed, read 'Sample preparation' on page 9.

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**Additional equipments or materials to be supplied by the user**

*Microcentrifuge*

*Sterile 1.5 ml microcentrifuge tubes*

*Water bath or heat block; 37°C and 65°C*

*Isopropanol*

*70% ethanol*

*Optional RNase solution (not provided)*

*\* Buffer AL and PP may precipitate at cool ambient temperature.  
If so, dissolve it in 37°C water bath.*

- 1. Transfer 900  $\mu$ l of Buffer RL to a fresh 1.5 ml microcentrifuge tube.**
- 2. Add 300  $\mu$ l of whole blood to the tube containing Buffer RL. Invert the tube 5~6 times to mix. Incubate the mixture for 10 min at room temperature.**

Invert 4~5 times during the incubation. The lysate should become translucent. If the lysate is opaque not translucent, it may be frozen or mis-stored sample, and you should resuspend the pellet and repeat step 2~3 with resuspended cells until lysate become translucent.

Do not incubate on ice or for more than 20 min.



- 3. Centrifuge for 30 sec at 14,000 x g. Carefully remove the supernatant as much as possible without disturbing the visible white (or pink) pellet. Resuspend the pellet in residual supernatant by vigorous vortexing or flicking.**

A little residual liquid will remain. Resuspending the cell pellet in residual liquid will greatly accelerate the efficiency of cell lysis at next step.

Steps 3~4 are critical steps for DNA recovery yield, so you have to check the translucent lysate and the white (or pink) pellet before processing next step.

- 4. Add 300  $\mu$ l of Buffer AL and pipet 5~6 times to resuspend thoroughly. Incubate the lysate at 37°C until clumps of cells disappear.**

Generally, cell lysis is completed in 5 min. Complete resuspending is crucial for good yield. If the clumps are still visible after 1 hour, add an additional 100  $\mu$ l of Buffer AL and repeat incubation.

- 5. (Optional :) If RNA-free DNA is required, add 1  $\mu$ l of RNase solution (20 mg/ml) to the lysate and mix the sample by inverting the tube 5 times. Incubate the mixture for 15 min at 37°C.**

- 6. Cool the sample to room temperature. Apply 100  $\mu$ l of Buffer PP to the mixture and vortex vigorously for 15 sec. Centrifuge for 2 min at 14,000 x g.**

**(Optional :) Incubate the sample on ice for 5 min before centrifugation. This may slightly increase the quality of DNA.**

A dark brown protein pellet should be visible.

- 7. Carefully transfer the supernatant to a fresh 1.5 ml micro centrifuge tube containing 300  $\mu$ l of isopropanol (room temperature) and gently mix the solution by inversion until the white thread-like strands of DNA form a visible mass.**

Be careful not to cotransfer the debris together.

If necessary, add glycogen or tRNA such as nucleic acid carrier before addition of isopropanol (Refer to 'DNA precipitation' on page 11).

Do not vortex after addition of isopropanol.

8. Centrifuge at 14,000 x g for 1 min. Decant the supernatant and add 300  $\mu$ l of 70% ethanol (room temperature). Gently invert the tube several times to wash the DNA pellet and the side walls of the tube.



A

9. Centrifuge at 14,000 x g for 1 min. Carefully discard the ethanol by aspirating or pipetting. Invert the tube on clean absorbent paper and air-dry the pellet for 10~15 min.

The DNA pellet is very loose at this point and care must be taken to avoid missing the pellet.

Ethanol should be completely removed, but over-dry will make the rehydration of DNA pellet difficult.

10. Add 100  $\mu$ l of Buffer RE or distilled water and rehydrate the DNA by incubating at 65°C for 1 hour.

During incubation, periodically mix the DNA solution by gently tapping the tube. DNA can be rehydrated alternatively by incubating the solution overnight at RT or 4°C.

## Protocol for 3 ml of Whole Blood



**B**

### [ GenEx™ Blood kit ]

Before proceed, read 'Sample preparation' on page 9.

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#### **Additional equipments or materials to be supplied by the user**

Centrifuge capable of handling of 15 ml tube

Sterile 15 ml centrifuge tubes

Water bath or heat block; 37°C and 65°C

Isopropanol

70% ethanol

Optional RNase solution (not provided)

\* Buffer AL may precipitate at cool ambient temperature.

If so, dissolve it in 37°C water bath.

- 1. Transfer 9 ml of Buffer RL to a fresh 15 ml centrifuge tube.**
- 2. Add 3 ml of whole blood to the tube containing Buffer RL. Invert the tube 5~6 times to mix. Incubate the mixture for 10 min at room temperature.**

Invert 4~5 times during the incubation. The lysate should become translucent. If the lysate is opaque not translucent, it may be frozen or mis-stored sample, and you should resuspend the pellet and repeat step 2~3 with resuspended cells until lysate become translucent.

Do not incubate on ice or for more than 20 min.

- 3. Centrifuge for 3 min at 2,000 x g. Carefully remove the supernatant as much as possible without disturbing the visible white (or pink) pellet. Resuspend the pellet in residual supernatant by vigorous vortexing or flicking.**

A little residual liquid will remain. Resuspending the cell pellet in residual liquid will greatly accelerate the efficiency of cell lysis at next step.

Steps 3~4 are critical steps for DNA recovery yields, so you have to check the translucent lysate and the white (or pink) pellet before processing next steps.

- 4. Add 3 ml of Buffer AL and pipet 5~6 times to resuspend thoroughly. Incubate the lysate at 37°C until clumps of cells disappear.**

Generally, cell lysis is completed in 5 min. Complete resuspending is crucial for good yield. If the clumps are still visible after 1 hour, add additional 1 ml of Buffer AL and repeat incubation.

- 5. (Optional :) If RNA-free DNA is required, add 6  $\mu$ l of RNase solution (20 mg/ml) to the lysate and mix the sample by inverting the tube 5 times. Incubate the mixture for 15 min at 37°C.**

- 6. Cool the sample to room temperature. Apply 1 ml of Buffer PP to the mixture and vortex vigorously for 15 sec. Centrifuge at 2,000 x g for 5 min.**

**(Optional :) Incubate the sample on ice for 5 min before centrifugation. This may slightly increase the quality of DNA.**

A dark brown protein pellet should be visible.

- 7. Carefully transfer the supernatant to a fresh 15 ml centrifuge tube containing 3 ml of isopropanol (room temperature) and gently mix the solution by inversion until the white thread-like strands of DNA form a visible mass.**

Be careful not to cotransfer the debris together.

Do not vortex after addition of isopropanol.



B

**8. Centrifuge at 2,000 x g for 3 min. Decant the supernatant carefully and add 3 ml of 70% ethanol (room temperature). Gently invert the tube several times to wash the DNA pellet and the side walls of the tube.**

**9. Centrifuge at 2,000 x g for 2 min. Carefully discard the ethanol by aspirating or pipetting. Invert the tube on clean absorbent paper and air-dry the pellet for 10~15 min.**

The DNA pellet is very loose at this point and care must be taken to avoid missing the pellet.

Ethanol should be completely removed, but over-dry will make the rehydration of DNA pellet difficult.

**10. Add 250  $\mu$ l of Buffer RE or distilled water and rehydrate the DNA by incubating at 65°C for 1 hour.**

During incubation, periodically mix the DNA solution by gently tapping the tube. DNA can be rehydrated alternatively by incubating the solution overnight at RT or 4°C.

**C**

## Protocol for 10 ml of Whole Blood

### [ *GenEx*<sup>™</sup> Blood kit ]

Before proceed, read 'Sample preparation' on page 9.

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#### ***Additional equipments or materials to be supplied by the user***

*Centrifuge capable of handling of 50 ml tube*

*Sterile 50 ml centrifuge tubes*

*Water bath or heat block; 37°C and 65°C*

*Isopropanol*

*70% ethanol*

*Optional RNase solution (not provided)*

*\* Buffer AL may precipitate at cool ambient temperature.*

*If so, dissolve it in 37°C water bath.*

- 1. Transfer 30 ml of Buffer RL to a fresh 50 ml centrifuge tube.**
- 2. Add 10 ml of whole blood to the tube containing Buffer RL. Invert the tube 5~6 times to mix. Incubate the mixture for 10 min at room temperature.**

Invert 4~5 times during the incubation.

The lysate should become translucent. If the lysate is opaque not translucent, it may be frozen or mis-stored sample, and you should resuspend the pellet and repeat step 2~3 with resuspended cells until lysate become translucent.

Do not incubate on ice or for more than 20 min.



- 3. Centrifuge for 5 min at 2,000 x g. Carefully remove the supernatant as much as possible without disturbing the visible white (or pink) pellet. Resuspend the pellet in residual supernatant by vigorous vortexing or flicking.**

Approximately several hundreds microliter of residual liquid will remain. Resuspending the cell pellet in residual liquid will greatly accelerate the efficiency of cell lysis at next step.

Steps 3~4 are critical steps for DNA recovery yields, so you have to check the translucent lysate and the white (or pink) pellet before processing next steps.

- 4. Add 10 ml of Buffer AL and pipet 5~6 times to resuspend thoroughly.**

**Incubate the lysate at 37°C until clumps of cells disappear.**

Generally, cell lysis is completed in 5 min. Complete resuspending is crucial for good yield. If the clumps are still visible after 1 hour, add additional 3 ml of Buffer AL and repeat incubation.

- 5. (Optional :) If RNA-free DNA is required, add 20  $\mu$ l of RNase solution (20 mg/ml) to the lysate and mix the sample by inverting the tube 4 times. Incubate the mixture for 15 min at 37°C.**

- 6. Apply 3.3 ml of Buffer PP to the mixture and vortex vigorously for 15 sec. Centrifuge at 2,000 x g for 5 min.**

**(Optional :) Incubate the sample on ice for 5 min before centrifugation. This may slightly increase the quality of DNA.**

If additional Buffer AL has been added at step 4, apply 4 ml of Buffer PP instead of 3.3 ml.

A dark brown protein pellet should be visible.

- 7. Carefully transfer the supernatant to a fresh 50 ml centrifuge tube containing 10 ml of isopropanol (room temperature) and gently mix the solution by inversion until the white thread-like strands of DNA form a visible mass.**

Be careful not to cotransfer the debris together.

Do not vortex after addition of isopropanol.

- 8. Centrifuge at 2,000 x g for 3 min. Decant the supernatant carefully and add 10 ml of 70% ethanol (room temperature). Gently invert the tube several times to wash the DNA pellet and the side walls of the tube.**

DNA will be visible as a small white pellet.

- 
- 9. Centrifuge at 2,000 x g for 2 min. Carefully discard the ethanol by aspirating or pipetting. Invert the tube on clean absorbent paper and air-dry the pellet for 10~15 min.**

The DNA pellet is very loose at this point and care must be taken to avoid missing the pellet.

Ethanol should be completely removed, but over-dry will make the rehydration of DNA pellet difficult.

- 10. Add 800  $\mu$ l of Buffer RE or distilled water and rehydrate the DNA by incubating at 65°C for 1 hour.**

During incubation, periodically mix the DNA solution by gently tapping the tube. DNA can be rehydrated alternatively by incubating the solution overnight at RT or 4°C.

## PROTOCOL for Buffy Coat Prepared from 3 ml of Whole Blood



D

### [ GenEx™ Blood kit ]

Before proceed, read 'Sample preparation' on page 9.

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#### **Additional equipments or materials to be supplied by the user**

Centrifuge capable of handling of 15 ml tube

Sterile 15 ml centrifuge tubes

Water bath or heat block; 37°C and 65°C

Isopropanol

70% ethanol

Optional RNase solution (not provided)

\* Buffer AL may precipitate at cool ambient temperature.

If so, dissolve it in 37°C water bath.

**1. Add 150~250  $\mu$ l buffy coat prepared from 3 ml of whole blood to a 15 ml centrifuge tube containing 3 times of Buffer RL.**

For example, mix 250  $\mu$ l buffy coat sample with 750  $\mu$ l Buffer RL.

Usually 150~250  $\mu$ l of buffy coat will be prepared from 3 ml of whole blood.

**2. Invert the tube 5~6 times to mix. Incubate the mixture for 10 min at room temperature.**

Invert 4~5 times during the incubation. Do not incubate sample mixture on ice or for more than 20 min.

**3. Continue with step 3 of 3 ml of Whole Blood protocol **B** (Page 18).**

  
**E**

## PROTOCOL for Cultured Cells ( $\sim 2 \times 10^6$ cells)

### [ GenEx™ Cell kit ]

Before proceed, read 'Sample preparation' on page 10.

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#### **Additional equipments or materials to be supplied by the user**

Microcentrifuge

Sterile 1.5 ml centrifuge tubes

Water bath or heat block ; 37°C and 65°C

Ice

Isopropanol, 70% ethanol

\* Buffer AL may precipitate at cool ambient temperature.

If so, dissolve it in 37°C water bath.

- 1. Harvest up to  $2 \times 10^6$  cells to a 1.5 ml fresh microcentrifuge tube by centrifugation at 14,000 x g for 10 sec. Discard the supernatant as much as possible.**

For adherent cells, treat trypsin-EDTA for detaching the cells before harvesting.

- 2. Resuspend the cell pellet in residual supernatant by vigorous vortexing or flicking.**

Complete resuspending is crucial for efficient lysis of cells.

Certain cells, such as PC12, do not lyse well in Buffer AL. For those cells, perform additional freeze-thaw step several times before proceeding to next step.

- 3. Add 300  $\mu$ l of Buffer AL and pipet to lyse the cells until no visible cell clumps remain.**

Usually the incubation time is not required. But if the clumps are still visible after pipetting, incubate at 37°C until the mixture becomes homogeneous.



- 4. Add 1  $\mu$ l of RNase solution (20 mg/ml) to the lysate and mix the sample by inverting the tube 5 times. Incubate the mixture for 5 min at 37°C.**
- 5. Cool the sample to room temperature. Add 100  $\mu$ l of Buffer PP to the mixture and vortex vigorously for 20 sec. Chill the sample on ice for 5 min.**

- 6. Centrifuge at 14,000 x g for 1 min.**  
A tight white protein pellet should be visible.

- 7. Carefully transfer the supernatant to a fresh 1.5 ml micro centrifuge tube containing 300  $\mu$ l of isopropanol (room temperature) and gently mix the solution by inversion until the white thread-like strands of DNA form a visible mass.**

Be careful not to cotransfer the debris together.

If necessary, add glycogen or tRNA as nucleic acid carrier before addition of isopropanol. (Refer to 'DNA precipitation' on page 11)

Do not vortex after addition of isopropanol.

- 8. Centrifuge at 14,000 x g for 1 min. Decant the supernatant and add 300  $\mu$ l of 70% ethanol (room temperature). Gently invert the tube several times to wash the DNA pellet and the side walls of the tube.**

- 9. Centrifuge at 14,000 x g for 1 min. Carefully discard the ethanol by aspirating or pipetting. Invert the tube on clean absorbent paper and air-dry the pellet for 10~15 min.**

The DNA pellet is very loose at this point and care must be taken to avoid missing the pellet.

Ethanol should be completely removed, but over-dry will make the rehydration of DNA pellet difficult.

- 10. Add 50  $\mu$ l of Buffer RE or distilled water and rehydrate the DNA by incubating at 65°C for 1 hour.**

During incubation, periodically mix the DNA solution by gently tapping the tube. DNA can be rehydrated alternatively by incubating the solution overnight at RT or 4°C.

**F**

## Protocol for Cultured Cells ( $\sim 2 \times 10^7$ cells)

### [ GenEx™ Cell kit ]

Before proceed, read 'Sample preparation' on page 10.

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#### **Additional equipments or materials to be supplied by the user**

Centrifuge capable of handling of 15 ml tube

Sterile 15 ml centrifuge tubes

Water bath or heat block; 37°C and 65°C

Ice

Isopropanol, 70% ethanol

\* Buffer AL may precipitate at cool ambient temperature.

If so, dissolve it in 37°C water bath.

- 1. Harvest up to  $2 \times 10^7$  cells to a 15 ml fresh centrifuge tube by centrifugation at 1,000 x g for 2 min. Discard the supernatant as much as possible.**

100~200  $\mu$ l of residual liquid will remain. For adherent cells, treat trypsin-EDTA for detaching the cells before harvesting.

- 2. Resuspend the cell pellet in residual supernatant by vigorous vortexing or flicking.**

Complete resuspending is crucial for efficient lysis of cells.

Certain cells, such as PC12, do not lyse well in Buffer AL. For those cells, perform additional freeze-thaw step several times before proceeding to next step.

- 3. Add 3 ml of Buffer AL and pipet to lyse the cells until no visible cell clumps remain.**

Usually the incubation time is not required. But if the clumps are still visible after pipetting, incubate at 37°C until the mixture becomes homogeneous.

- 4. Add 6  $\mu$ l of RNase solution (20 mg/ml) to the lysate and mix the sample by inverting the tube 5 times. Incubate the mixture for 5 min at 37°C.**
- 5. Cool the sample to room temperature. Add 1 ml of Buffer PP to the mixture and vortex vigorously for 20 sec. Chill the sample on ice for 5 min.**
- 6. Centrifuge at 2,000 x g for 10 min.**

A tight white protein pellet should be visible.
- 7. Carefully transfer the supernatant to a fresh 15 ml centrifuge tube containing 3 ml of isopropanol (room temperature) and gently mix the solution by inversion until the white thread-like strands of DNA form a visible mass.**

Be careful not to cotransfer the debris together.  
Do not vortex after addition of isopropanol.
- 8. Centrifuge at 2,000 x g for 3 min. Decant the supernatant and add 3 ml of 70% ethanol (room temperature). Gently invert the tube several times to wash the DNA pellet and the side walls of the tube.**
- 9. Centrifuge at 2,000 x g for 1 min. Carefully discard the ethanol by aspirating or pipetting. Invert the tube on clean absorbent paper and air-dry the pellet for 10~15 min.**

The DNA pellet is very loose at this point and care must be taken to avoid missing the pellet.  
Ethanol should be completely removed, but over-dry will make the rehydration of DNA pellet difficult.
- 10. Add 250  $\mu$ l of Buffer RE or distilled water and rehydrate the DNA by incubating at 65°C for 1 hour.**

During incubation, periodically mix the DNA solution by gently tapping the tube. DNA can be rehydrated alternatively by incubating the solution overnight at RT or 4°C.



## Protocol for Gram Negative Bacteria

### [ GenEx™ Cell kit ]

Before proceed, read 'Sample preparation' on page 10.

#### **Additional equipments or materials to be supplied by the user**

*Microcentrifuge*

*Sterile 1.5 ml centrifuge tubes*

*50 mM EDTA, pH 8.0*

*Water bath or heat block ; 37°C, 65°C and 80°C*

*Ice*

*Isopropanol and 70% ethanol (RT)*

*\* Buffer AL may precipitate at cool ambient temperature.*

*If so, dissolve it in 37°C water bath.*

- 1. Add up to  $1 \times 10^9$  of bacterial cells to a 1.5 ml micro centrifuge tube.**  
When  $OD_{600} = 1$ , the cell density may be  $1 \times 10^9$  cells per milliliter approximately.
- 2. Centrifuge at  $14,000 \times g$  for 1 min to pellet the cells. Remove the supernatant.**
- 3. Add  $300 \mu\text{l}$  of Buffer AL and gently pipet until the cells are resuspended thoroughly.**
- 4. Incubate at  $80^\circ\text{C}$  for 5 min. Cool to room temperature.**  
This step is especially necessary for pathogenic bacterial strains.
- 5. Add  $1 \mu\text{l}$  of RNase Solution (20 mg/ml). Invert the tube 2~5 times to mix. Incubate at  $37^\circ\text{C}$  for 15~60 min.**



**6. Cool the sample to room temperature. Add 100  $\mu$ l of Buffer PP and vortex vigorously for 20 sec. Incubate on ice for 5 min.**

**7. Centrifuge at 14,000 x g for 3 min.**

**8. Carefully transfer the supernatant to a fresh 1.5 ml microcentrifuge tube containing 300  $\mu$ l of isopropanol (room temperature) and gently mix the solution by inversion until the white thread-like strands of DNA form a visible mass.**

Be careful not to cotransfer the debris together.

Do not vortex after addition of isopropanol.

**9. Centrifuge at 14,000 x g for 1 min. Decant the supernatant and add 300  $\mu$ l of 70% ethanol (room temperature). Gently invert the tube several times to wash the DNA pellet and the side walls of the tube.**

**10. Centrifuge at 14,000 x g for 1 min. Carefully discard the ethanol by aspirating or pipetting. Invert the tube on clean absorbent paper and air-dry the pellet for 10~15 min.**

The DNA pellet is very loose at this point and care must be taken to avoid missing the pellet.

Ethanol should be completely removed, but over-dry will make the rehydration of DNA pellet difficult.

**11. Add 100  $\mu$ l of Buffer RE or distilled water and rehydrate the DNA by incubating at 65°C for 1 hour.**

During incubation, periodically mix the DNA solution by gently tapping the tube. Alternatively, DNA can be rehydrated by incubating the solution overnight at RT or 4°C.

  
**H**

# PROTOCOL for Animal Tissue

## [ GenEx™ Tissue kit ]

Before proceed, read 'Sample preparation' on page 10.

### **Additional equipments or materials to be supplied by the user**

Microcentrifuge

Small homogenizer, sharp blade or mortar and pestle, liquid nitrogen

Sterile 1.5 ml microcentrifuge tubes

Water bath or heat block ; 37°C, 56°C and 65°C

Ice

Isopropanol, 70% ethanol

\* Buffer AL may precipitate at cool ambient temperature.

If so, dissolve it in 37°C water bath.

- 1. Homogenize up to 10 mg of tissue in 300  $\mu$ l of Buffer AL using small homogenizer. Transfer the lysate to a fresh 1.5 ml microcentrifuge tube. Proceed to step 2.**

Carefully homogenize the sample tissue not to foam if possible.

**Alternative 1 :** Grind sample tissue in liquid nitrogen with pre-chilled mortar and pestle. After grinding, let the liquid nitrogen evaporate and add up to 10 mg of tissue to 1.5 ml microcentrifuge tube containing 300  $\mu$ l of Buffer AL. Proceed to step 2.

**Alternative 2 :** Mince up to 10 mg of tissue sample as small as possible and put it into 1.5 ml microcentrifuge tube containing 300  $\mu$ l of Buffer AL. Incubate for 10 min at 65°C. Homogenize flabby sample tissue with small homogenizer.

- 2. Add 1.5  $\mu$ l of Proteinase K (20 mg/ml) to the lysate and mix the sample by vortexing or inverting. Incubate the mixture at 56°C until the sample is completely lysed. It may take about 10 min~overnight depending on the sample type.**

The lysate should become translucent without any particles after complete lysis.

- 3. Add 1  $\mu$ l of RNase solution (20 mg/ml) to the lysate and mix the sample by inverting the tube 5 times. Incubate the mixture for 15~30 min at 37°C.**
- 4. Cool the sample to room temperature. Add 100  $\mu$ l of Buffer PP to the mixture and vortex vigorously for 20 sec. Chill the sample on ice for 5 min.**

- 5. Centrifuge at 14,000 x g for 1 min.**

A tight white protein pellet should be visible.

- 6. Carefully transfer the supernatant to a fresh 1.5 ml micro centrifuge tube containing 300  $\mu$ l of isopropanol (room temperature) and gently mix the solution by inversion until the white thread-like strands of DNA form a visible mass.**

Be careful not to cotransfer the debris together.

If necessary, add glycogen or tRNA as nucleic acid carrier before addition of isopropanol. (Refer to 'DNA precipitation' on page 11)

Do not vortex after addition of isopropanol.

- 7. Centrifuge at 14,000 x g for 1 min. Decant the supernatant and add 300  $\mu$ l of 70% ethanol (room temperature). Gently invert the tube several times to wash the DNA pellet and the side walls of the tube.**

- 8. Centrifuge at 14,000 x g for 1 min. Carefully discard the ethanol by aspirating or pipetting. Invert the tube on clean absorbent paper and air-dry the pellet for 10~15 min.**

The DNA pellet is very loose at this point and care must be taken to avoid missing the pellet. Ethanol should be completely removed, but over-dry will make the rehydration of DNA pellet difficult.

- 9. Add 100  $\mu$ l of Buffer RE or distilled water and rehydrate the DNA by incubating at 65°C for 1 hour.**

When starting sample is buccal swab (page 34) or body fluids (page 35), use less volume (10~20  $\mu$ l) of Buffer RE for rehydration. During incubation, periodically mix the DNA solution by gently tapping the tube. DNA can be rehydrated alternatively by incubating the solution overnight at RT or 4°C.



  
I

## Protocol for Paraffin-Embedded Tissue

### [ GenEx™ Tissue kit ]

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#### **Additional equipments or materials to be supplied by the user**

Microcentrifuge

Sterile 1.5 ml microcentrifuge tubes

Water bath or heat block ; 37°C, 56°C and 65°C

Ice

Xylene, Isopropanol, Absolute ethanol, 70% ethanol

\* Buffer AL may precipitate at cool ambient temperature.

If so, dissolve it in 37°C water bath.

1. Place 5~10 mg of paraffin-fixed tissue in a fresh 1.5 ml micro centrifuge tube. Add 300  $\mu$ l Xylene and incubate 5 min with constant mixing gently at room temperature.
2. Centrifuge at 14,000 x g for 3 min. Carefully remove supernatant by pipetting.
3. Repeat step 1~2 twice.
4. Add 300  $\mu$ l of absolute ethanol and incubate 5 min with constant mixing at room temperature.
5. Centrifuge at 14,000 x g for 3 min. Carefully remove supernatant by pipetting.

6. Repeat step 4~5 twice.
7. Add 300  $\mu$ l Buffer AL and homogenize using 30~50 strokes with a microcentrifuge tube pestle.  
Carefully homogenize the sample not to foam if possible.
8. Add 1.8  $\mu$ l of Proteinase K solution (20 mg/ml) to the lysate, mix by inverting.
9. Incubate at 56°C for 3 hours to complete lysis.  
Invert the sample periodically during the incubation.
10. Continue with step 3 of Animal Tissue protocol **H** (Page 31).



  
**J**

## Protocol for Buccal Swab

### [ GenEx™ Cell/Tissue kit ]

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#### **Additional equipments or materials to be supplied by the user**

Microcentrifuge

Sterile 1.5 ml microcentrifuge tubes

Water bath or heat block ; 37°C, 56°C and 65°C

Buccal swab, wire cutter, tweezer

Ice

Isopropanol, 70% ethanol

\* Buffer AL may precipitate at cool ambient temperature.

If so, dissolve it in 37°C water bath.

- 1. Add 300 µl Buffer AL to a fresh 1.5 ml microcentrifuge tube and place brush into the tube. Clip off handle of brush with wire cutters so tube can be closed.**

Cutters should be rinsed with 70% ethanol between samples to prevent contamination.

- 2. Incubate at 65°C for 15~60 min.**

If maximum yield is required, add 1.8 µl Proteinase K solution (20 mg/ml) and incubate at 56°C for 1 hour.

- 3. Remove brush with tweezers.**

Tweezers should be rinsed with 70% ethanol between samples to prevent contamination.

- 4. Continue with step 3 of Animal Tissue protocol **H** (Page 31).**

## [ GenEx™ Cell/Tissue kit ]

### **Additional equipments or materials to be supplied by the user**

Microcentrifuge

Sterile 1.5 ml microcentrifuge tubes

Water bath or heat block ; 37°C, 56°C and 65°C

Ice

Isopropanol

70% ethanol

\* Buffer AL may precipitate at cool ambient temperature.

If so, dissolve it in 37°C water bath.

- 1. Add 50 µl body fluid (e.g. cerebrospinal fluid, plasma, serum, saliva, various mucous discharges, synovial fluids, and etc.) to a fresh 1.5 ml microcentrifuge tube containing 250 µl Buffer AL. Pipet up and down to mix thoroughly.**

Body fluids usually contain very low concentration of cells. To concentrate sample, centrifuge at 2,000 x g for 10 min and remove supernatant leaving behind desired volume of residual liquid. Resuspend thoroughly the cell pellet with residual liquid and place on ice before use.

- 2. Incubate at 65°C for 15 min.**

If maximum yield is required, add 1.8 µl Proteinase K solution (20 mg/ml) and incubate at 56°C for 1 hour.

- 3. Continue with step 3 of Animal Tissue protocol **H** (Page 31).**

L

## Protocol for Mouse Tail

### [ GenEx™ Tissue kit ]

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#### **Additional equipments or materials to be supplied by the user**

Microcentrifuge

Sterile sharp blade

Sterile 1.5 ml microcentrifuge tubes

Water bath or heat block ; 37°C, 56°C and 65°C

Ice

Isopropanol, 70% ethanol

\* Buffer AL may precipitate at cool ambient temperature.

If so, dissolve it in 37°C water bath.

- 1. Mince 0.5~1 cm of mouse tail as small as possible. Transfer it to the 1.5~2 ml microcentrifuge tube containing 600 µl of Buffer AL.**
- 2. Add 1.8 ul of Proteinase K solution (20 mg/ml).**
- 3. Incubate overnight at 56°C with gentle shaking.**  
Alternatively, incubate for 3 hours at 56°C; vortex the sample once or twice per hour during 3-hours incubation. Make sure the tail is completely digested.
- 4. Add 1 µl of RNase solution (20 mg/ml) to the lysate and mix the sample by inverting the tube 5 times. Incubate the mixture for 15~30 min at 37°C.**

**5. Cool the sample to room temperature. Add 200  $\mu\text{l}$  of Buffer PP to the mixture and vortex vigorously for 20 sec. Chill the sample on ice for 5 min.**

**6. Centrifuge at 14,000 x g for 1 min.**

A tight white protein pellet should be visible.

**7. Carefully transfer the supernatant to a fresh 1.5~2 ml micro centrifuge tube containing 600  $\mu\text{l}$  of isopropanol (room temperature) and gently mix the solution by inversion until the white thread-like strands of DNA form a visible mass.**

Be careful not to cotransfer the debris together.

Do not vortex after addition of isopropanol.

**8. Centrifuge at 14,000 x g for 1 min. Decant the supernatant and add 600  $\mu\text{l}$  of 70% ethanol (room temperature). Gently invert the tube several times to wash the DNA pellet and the side walls of the tube.**

**9. Centrifuge at 14,000 x g for 1 min. Carefully discard the ethanol by aspirating or pipetting. Invert the tube on clean absorbent paper and air-dry the pellet for 10~15 min.**

The DNA pellet is very loose at this point and care must be taken to avoid missing the pellet.

Ethanol should be completely removed, but over-dry will make the rehydration of DNA pellet difficult.

**10. Add 50  $\mu\text{l}$  of Buffer RE or distilled water and rehydrate the DNA by incubating at 65°C for 1 hour.**

During incubation, periodically mix the DNA solution by gently tapping the tube. DNA can be rehydrated alternatively by incubating the solution overnight at RT or 4°C.





## Troubleshooting Guide

Facts	Possible Causes	Suggestions
<b>Low or no yield</b>	Starting material is too old or mis-stored	Best yield will be obtained from fresh sample. DNA yield is dependent on the type, size, age and storage of starting material. Lower yield will be obtained from material that has been inappropriately stored. For example, blood samples that have been stored at 4°C for more than 5 days may bring about reduced yield. Refer to 'Sample preparation' on Page 9~10.
	Low cells in the sample	Some sample may contain low concentration of nucleated cells, and this may lead to poor yield. Increase the sample amount. If possible, harvest new sample and repeat the DNA purification with new sample.
	Insufficient lysis	Incomplete lysis can be due to too much starting material. Add more Buffer AL to completely lyse the cells. Start with proper amount of sample material. For cultured cells or bacteria, starting cell numbers should be determined with cell counter.
	White blood cell pellet was not resuspend thoroughly in step 3 of protocol A, B, C	The white blood cell pellet must be vortexed vigorously to resuspend the cells thoroughly.
	Lost DNA pellet during isopropanol precipitation	Intensive care must be taken in removing the isopropanol or ethanol not to lose the pellet.
	Cell clumps present in the lysate	Cell clumps will remain until cells are completely lysed. Incomplete lysis of cells will bring about poor yield. To lyse completely the cells in the clumps, incubate sample at either 37°C or room temperature with periodic mixing until the solution is homogeneous.



Facts	Possible Causes	Suggestions
<b>Low or no yield</b>	DNA pellet is not completely rehydrated	Rehydrate DNA by incubating at 65°C for 1 hour. During incubation, periodically mix the DNA solution by gently tapping the tube. Alternatively, DNA can be rehydrated by incubating the solution overnight at RT or 4°C
<b>Degraded DNA</b>	Starting material is too old or mis-stored	Too old or mis-stored sample often yield degraded DNA. Use fresh sample.
<b>No protein pellet</b>	Lysate does not sufficiently cooled down.	To obtain a tight protein pellet, the sample should be cooled to room temperature or chilled on ice 5 min before adding Buffer PP. After addition of Buffer PP, vortex vigorously for complete mixing.
<b>DNA pellet difficult to dissolve</b>	Over-dried pellet	DNA pellets should not be dried for longer than 15 min at room temperature. Rehydrate DNA by incubating for 1 hour at 65°C and then leave the sample at room temperature or 4°C overnight. DO NOT leave DNA at 65°C overnight. This may degrade DNA.



# APPENDIX

## A.

### *Protocol for* Large Scale Cultured Cell

1. Harvest up to  $1 \times 10^8$  cells to a 50 ml fresh centrifuge tube by centrifugation at  $1,000 \times g$  for 2 min. Discard the supernatant as much as possible.
2. Resuspend the cell pellet in residual supernatant by vigorous vortexing or flicking.
3. Add 15 ml of Buffer AL and pipet to lyse the cells until no visible cell clumps remain.
4. Add  $30 \mu\text{l}$  of RNase A (20 mg/ml) and incubation 5 min at  $37^\circ\text{C}$ .
5. Add 5 ml of Buffer PP and vortex for 20 sec. Chill the sample on ice for 5 min.
6. Centrifuge at  $2,000 \times g$  for 5 min.
7. Transfer the supernatant to a fresh 50 ml centrifuge tube containing 15 ml of isopropanol and gently mix the solution by inversion.
8. Centrifuge at  $2,000 \times g$  for 3 min. Decant the supernatant and add 15 ml of 70% ethanol.  
Gently invert the tube several times to wash the DNA pellet and side walls of the tube.
9. Centrifuge at  $2,000 \times g$  for 2 min. Carefully discard the ethanol by aspirating or pipetting.  
Invert the tube on clean absorbent paper and air-dry the pellet for 10~15 min.
10. Add 1 ml of Buffer RE or distilled water and rehydrate the DNA by incubating at  $65^\circ\text{C}$  for 1 hour.

# APPENDIX

## B.

### *Protocol for* Large Scale Tissue

1. Homogenize up to 100 mg of tissue in 3 ml of Buffer AL.
2. Transfer the lysate to a fresh 15 ml centrifuge tube.
3. Add 15  $\mu$ l of Proteinase K (20 mg/ml) to the lysate and mix the sample by vortexing or inverting. Incubate the mixture at 56°C until the sample is completely lysed.  
It may take about 10 min~overnight depending on the sample type.  
The lysate should become translucent without any particles after complete lysis.
4. Add 6  $\mu$ l of RNase A (20 mg/ml) and incubation 15~30 min at 37°C.
5. Add 1 ml of Buffer PP and vortex for 20 sec. Chill the sample on ice for 5 min.
6. Centrifuge at 2,000 x g for 5 min.
7. Transfer the supernatant to a fresh 15 ml centrifuge tube containing 3 ml of isopropanol and gently mix the solution by inversion.
8. Centrifuge at 2,000 x g for 3 min. Decant the supernatant and add 3 ml of 70% ethanol.  
Gently invert the tube several times to wash the DNA pellet and side walls of the tube.
9. Centrifuge at 2,000 x g for 2 min. Carefully discard the ethanol by aspirating or pipetting.  
Invert the tube on clean absorbent paper and air-dry the pellet for 10~15 min.
10. Add 600  $\mu$ l of Buffer RE or distilled water and rehydrate the DNA by incubating at 65°C for 1 hour.

# APPENDIX

## C.

### *Protocol for* Removal of RNA from Purified DNA

1. Add 1  $\mu$ l of RNase solution per 100  $\mu$ l DNA solution. Incubate the mixture for 15~30 min at 37°C.
2. Add 0.5 volumes of Buffer PP and 1 volumes of isopropanol to the DNA sample and gently mix the solution by inversion.
3. Centrifuge at 14,000  $\times$  g for 1 min (micro centrifuge tube) or for 3 min at 2,000  $\times$  g (15 or 50 ml centrifuge tube).
4. Decant the supernatant and add 2 volumes of 70% ethanol. Gently invert the tube several times to wash the DNA pellet and side walls of the tube.
5. Centrifuge at 14,000  $\times$  g for 1 min (micro centrifuge tube) or for 2 min at 2,000  $\times$  g (15 or 50 ml centrifuge tube).
6. Carefully discard the ethanol by aspirating or pipetting. Invert the tube on clean absorbent paper and air dry the pellet for 10~15 min.
7. Add 1 volumes of Buffer RE or distilled water and rehydrate the DNA by incubating at 65°C for 1 hour.

# Ordering Information

Products	Scale	Size	Cat. No.	Type
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## GeneAll® Hybrid-Q™ for rapid preparation of plasmid DNA

Plasmid Rapidprep	mini	50	100-150	spin
		200	100-102	

## GeneAll® Exprep™ for preparation of plasmid DNA

Plasmid SV	mini	50	101-150	spin /
		200	101-102	vacuum
	Midi	26	101-226	spin /
50		101-250	vacuum	
		100	101-201	

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

Plasmid LE (Low Endotoxin)	mini	50	111-150	spin /
		200	111-102	vacuum
	Midi	26	111-226	spin /
100		111-201	vacuum	
Plasmid EF (Endotoxin Free)	Midi	20	121-220	spin
		100	121-201	

## GeneAll® Expin™ for purification of fragment DNA

Gel SV	mini	50	102-150	spin /
		200	102-102	vacuum
PCR SV	mini	50	103-150	spin /
		200	103-102	vacuum
CleanUp SV	mini	50	113-150	spin /
		200	113-102	vacuum
Combo GP	mini	50	112-150	spin /
		200	112-102	vacuum

## GeneAll® Exgene™ for isolation of total DNA

Tissue SV	mini	100	104-101	spin /
		250	104-152	vacuum
		26	104-226	spin /
	Midi	100	104-201	vacuum
		10	104-310	spin /
	26	104-326	vacuum	
Tissue plus! SV	mini	100	109-101	spin /
		250	109-152	vacuum
	Midi	26	109-226	spin /
		100	109-201	vacuum
	MAXI	10	109-310	spin /
		26	109-326	vacuum

Products	Scale	Size	Cat. No.	Type
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## GeneAll® Exgene™ for isolation of total DNA

Blood SV	mini	100	105-101	spin /
		250	105-152	vacuum
	Midi	26	105-226	spin /
		100	105-201	vacuum
	MAXI	10	105-310	spin /
		26	105-326	vacuum
Cell SV	mini	100	106-101	spin /
		250	106-152	vacuum
	MAXI	10	106-310	spin /
		26	106-326	vacuum
	mini	100	108-101	spin /
		250	108-152	vacuum
Clinic SV	Midi	26	108-226	spin /
		100	108-201	vacuum
	MAXI	10	108-310	spin /
		26	108-326	vacuum
Genomic DNA micro	mini	50	118-050	spin
		100	117-101	spin /
Plant SV	mini	250	117-152	vacuum
		26	117-226	spin /
	Midi	100	117-201	vacuum
		10	117-310	spin /
	MAXI	26	117-326	vacuum
		50	114-150	spin
Soil DNA mini	mini	50	115-150	spin
Stool DNA mini	mini	50	115-151	spin
Stool-Bead DNA mini	mini	50	128-150	spin
Viral DNA / RNA	mini	50	138-150	spin
FFPE Tissue DNA	mini	250	138-152	spin

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

GenEx™ Blood	Sx	100	220-101	solution
		500	220-105	
	Lx	100	220-301	solution
		100	221-101	
GenEx™ Cell	Sx	500	221-105	solution
		100	221-301	
GenEx™ Tissue	Sx	100	222-101	solution
		500	222-105	
	Lx	100	222-301	solution

Products	Scale	Size	Cat. No.	Type
<b>GeneAll® GenEx™</b> for isolation of total DNA				
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 preparation 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 preparation 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
Allspin™	mini	50	306-150	spin
RiboSaver™	mini	100	351-001	solution

Products	Scale	Size	Cat. No.	Type
<b>GeneAll® AmpONE™</b> 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	

Products	Scale	Size	Cat. No.	Type
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### GeneAll® Protein series

ProtinEx™ Animal cell / tissue	100 ml		701-001	solution
PAGESTA™ Reducing 5X SDS-PAGE Sample Buffer	1 ml × 10 tubes		751-001	solution

### GeneAll® STEADi™ for automatic nucleic acid purification

12 Instrument			GST012	system
24 Instrument			GST024	system
Genomic DNA Cell / Tissue	96		401-104	kit
Genomic DNA Blood	96		402-105	kit
Total RNA	96		404-304	kit
Viral DNA / RNA	96		405-322	kit
Genomic DNA Plant	96		407-117	kit
Soil DNA	96		408-114	kit

### GeneAll® GENTi™<sup>32</sup> *Ultimately flexible automatic extraction system*

Automatic extraction equipment			GT1032	system
Genomic DNA	48		901-048	tube
	96		901-096	plate
Viral DNA / RNA	48		902-048	tube
	96		902-096	plate
Whole Blood Genomic DNA	48		903-048	tube
	96		903-096	plate

Products	Scale	Size	Cat. No.	Type
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### GeneAll® GENTi™<sup>32</sup> *Ultimately flexible automatic extraction system*

Automatic extraction equipment			GT1032A	system
Genomic DNA	48		901-048A	tube
	96		901-096A	plate
Viral DNA / RNA	48		902-048A	tube
	96		902-096A	plate
Plant DNA / RNA	48		904-048A	tube
	96		904-096A	plate
Blood DNA	48		903-048A	tube
	96		903-096A	plate

**Note.**

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# GeneAll

GENEALL BIOTECHNOLOGY CO., LTD



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GeneAll Bldg., 303-7, Dongnamro, Songpa-gu, Seoul, Korea 05729

E-mail : [sales@geneall.com](mailto:sales@geneall.com)

Tel. 82-2-407-0096 Fax. 82-2-407-0779

[www.geneall.com](http://www.geneall.com)

Manufacturer site

A-1201 ~ A-1204, Hanam Techno Valley UI Center,  
947, Hanam-daero, Hanam-si, Gyeonggi-do, 12982, Korea