Ver 2.1

Handbook for

# GenEx™ Blood
# GenEx™ Cell
# GenEx™ Tissue

## TOTAL DNA PURIFICATION KIT

genex tm

GeneAll

## Customer & Technical Support

Do not hesitate to ask us any question.

We thank you for any comment or advice.

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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 )

Visit www.geneall.com or www.geneall.co.kr for FAQ, QnA and more information.

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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 ul | 600 ul | 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 ul | 600 ul | 600 ul |
| Protocol Handbook | 1 | 1 | 1 |

** On the basis of DNA purification from 300 ul whole blood, 2 x $10^6$ cells or 10 mg animal tissue*

*** On the basis of DNA purification from 10 ml whole blood, 1 x $10^8$ cells or 100 mg animal tissue*

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

## Product Disclaimer

GeneAll® *GenEx*™ 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*™ 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*™ 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 ul | 5 ~ 15 ug |
| | | 3 ml | 80 ~ 150 ug |
| | | 10 ml | 250 ~ 500 ug |
| | Mouse | 300 ul | 6 ~ 7 ug |
| Buffy coat* | Human | 150 ~ 250 ul | 50 ~ 150 ug |
| Body fluids | Human | 50 ul | 0.1 ~ 2.5 ug |
| Cultured cell lines | CHO | 2 x $10^6$ cells | 14 ~ 16 ug |
| | RAW264.7 | 2 x $10^6$ cells | 16 ~ 17 ug |
| | COS | 1.5 x $10^6$ cells | 9 ~ 12 ug |
| | K562 | 3 x $10^6$ cells | 15 ~ 30 ug |
| | NIH3T3 | 2 x $10^6$ cells | 9 ~ 13 ug |
| | PC12 | 8 x $10^6$ cells | 5 ~ 8 ug |
| Animal tissue | Mouse Liver | 10 mg | 20 ~ 25 ug |
| | Mouse Pancreas | 10 mg | 70 ~ 75 ug |
| | Mouse Heart | 10 mg | 2 ~ 4 ug |
| | Mouse Tail | 1 cm of tail tip | 15 ~ 30 ug |
| Gram (-) bacteria | E.Coli / JM109 | 2 x $10^9$ cells | 18 ~ 25 ug |
| | E.cloacae | 6 x $10^9$ cells | 20 ~ 26 ug |

** 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

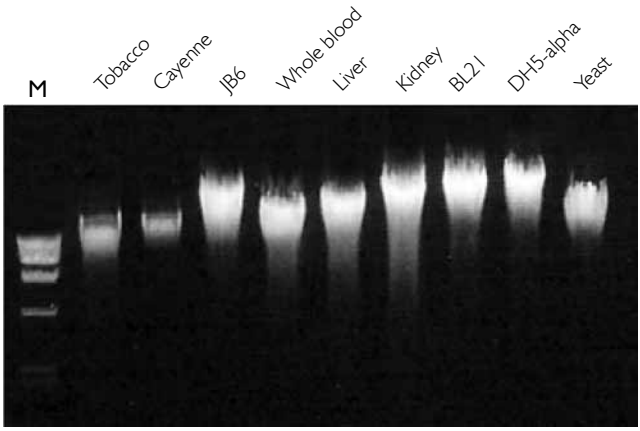
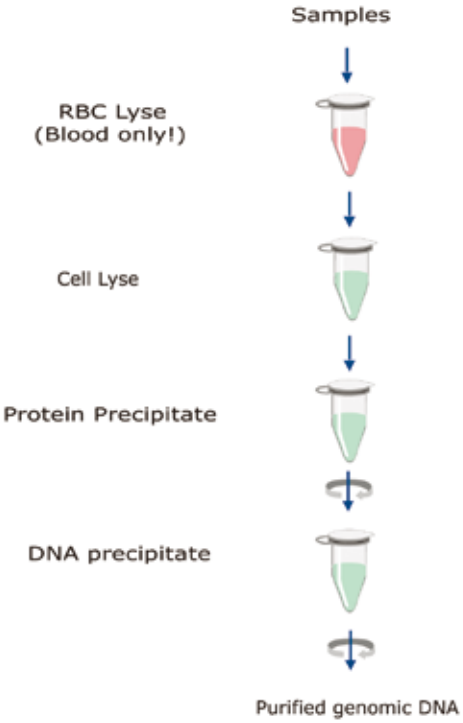


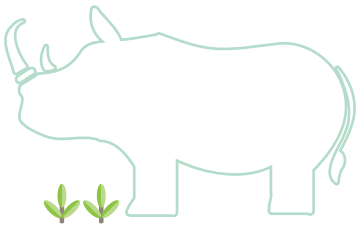

*Fig 1. Genomic DNA prepared from several kinds of organism using GenEx*™ *Genomic DNA purification kit. 5ul of eluate from each sample was resolved on 0.7% agarose gel.*

# 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 hematocytometer or other cell counter. 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 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 mortal 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°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°C or -80°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 $Na^+$, 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 (ul) | 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 (ul)* | 30 | 100 | 150 | 200 | 250 | 500 | 800 |
| DNA yield (ug)** | 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 (ul) | 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 (ul)* | 25 | 25 | 50 | 150 | 300 | 800 | 1000 |
| DNA yield (ug)** | 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 (ul) | 1 | 1.5 | 7.5 | 15 |
| RNase A (ul) | 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 (ul)* | 50 | 100 | 350 | 600 |
| DNA yield (ug)** | 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 ul of Whole Blood

## [ *GenEx*™ Blood kit ]

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

***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 ul of Buffer RL to a fresh 1.5 ml microcentrifuge tube.**

**2. Add 300 ul 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 xg. 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 ul 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 ul of Buffer AL and repeat incubation.

**5. *(Optional :)* If RNA-free DNA is required, add 1 ul 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 ul of Buffer PP to the mixture and vortex vigorously for 15 sec. Centrifuge for 2 min at 14,000 xg.**

***(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 ul 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 xg for 1 min. Decant the supernatant and add 300 ul 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 xg 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 ul 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.

## [ *GenEx*™ Blood kit ]

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

***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 xg. 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 ul 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 xg 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.

**8.** **Centrifuge at 2,000 xg 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.**

B

**9.** **Centrifuge at 2,000 xg 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 ul 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*™ Blood kit ]

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

***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 xg. 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 ul 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 xg 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 xg 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 xg 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 ul 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.

## [ *GenEx*™ Blood kit ]

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

***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 ul 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 ul buffy coat sample with 750 ul Buffer RL.
   Usually 150 ~ 250 ul of buffy coat will be prepared from 3ml 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 (~ 2 x $10^6$ cells)

## [ *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*
*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 x $10^6$ cells to a 1.5 ml fresh microcentrifuge tube by centrifugation at 14,000 xg 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 ul 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 ul 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 ul 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 xg 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 ul 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 xg for 1 min. Decant the supernatant and add 300 ul 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 xg 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 ul 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 (~ 2 x $10^7$ cells)

## [ *GenEx*™ Cell kit ]

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

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

100 ~ 200 ul 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 ul 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 xg 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 xg 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 xg 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 ul 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.

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

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

**7.** **Centrifuge at 14,000 xg for 3 min.**

**8.** **Carefully transfer the supernatant to a fresh 1.5 ml microcentrifuge tube containing 300 ul 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 xg for 1 min. Decant the supernatant and add 300 ul 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 xg 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 ul 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 ul 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 ul 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 ul of Buffer AL. Incubate for 10 min at 65°C. Homogenize flabby sample tissue with small homogenizer.**

**2. Add 1.5 ul 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 ul 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 ul 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 xg 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 ul 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 xg for 1 min. Decant the supernatant and add 300 ul 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 xg 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 ul 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 ul) 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 ]

***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, Alsolute 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 ul Xylene and incubate 5 min with constant mixing gently at room temperature.**

2. **Centrifuge at 14,000 xg for 3 min. Carefully remove supernatant by pipetting.**

3. **Repeat step 1 ~ 2 twice.**

4. **Add 300 ul of absolute ethanol and incubate 5 min with constant mixing at room temparature.**

5. **Centrifuge at 14,000 xg for 3 min. Carefully remove supernatant by pipetting.**

**6.** **Repeat step 4 ~ 5 twice.**

**7.** **Add 300 ul 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 ul 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 ]

***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 ul 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 ul 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 ul 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 ul 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 xg 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 ul 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 ]

***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 ul 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 ul 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 ul 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 xg 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 ul 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 xg for 1 min. Decant the supernatant and add 600 ul 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 xg 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 ul 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 |
|---|---|---|
| **Low or no yield** | 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 |
|---|---|---|
| **Low or no yield** | 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 |
| **Degraded DNA** | Starting material is too old or mis-stored | Too old or mis-stored sample often yield degraded DNA. Use fresh sample. |
| **No protein pellet** | 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. |
| **DNA pellet difficult to dissolve** | 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 x $10^8$ cells to a 50 ml fresh centrifuge tube by centrifugation at 1,000 xg 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 ul of RNase A (20 mg/ml) and incubation 5 min at 37°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 xg 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 xg 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 xg 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°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 ul 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 ul 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 xg 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 xg 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 xg 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 ul 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 ul of RNase solution per 100 ul 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 xg for 1 min (micro centrifuge tube) or for 3 min at 2,000 xg (15 ml 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 xg for 1 min (micro centrifuge tube) or for 2 min at 2,000 xg (15 ml 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.

## Note .

# Ordering Information

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

| Products | Scale | Size | Cat. No. | Type |
|---|---|---|---|---|
| Plasmid Rapidprep | | 50 | 100-150 | mini / spin |
| | | 100 | 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 | |
| | | 1,000 | 101-111 | |
| | Midi | 26 | 101-226 | spin / vacuum |
| | | 50 | 101-250 | |
| | | 100 | 101-201 | |

### GeneAll® *Exfection™*
*for preparation of highly pure 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 | mini | 50 | 114-150 | spin |
| GMO SV | mini | 50 | 107-150 | spin / vacuum |
| | | 200 | 107-102 | |

### GeneAll® *GenEx™* *for isolation of total DNA*

| 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*

| | | | | |
|---|---|---|---|---|
| *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 | 228-201 | |
| | Lx | 20 | 228-301 | |

### GeneAll® DirEx™
*for preperation of PCR-template without extraction*

| | | | | |
|---|---|---|---|---|
| DirEx™ | | 50 | 250-050 | 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 ™ vRD | mini | 50 | 302-150 | spin |
| Ribospin ™ vRD *plus!* | mini | 50 | 312-150 | spin |
| Ribospin ™ Plant | mini | 50 | 307-150 | spin |
| Allspin™ | mini | 50 | 306-150 | spin |

### 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 | |
| α-Taq DNA polymerase | 250 U | 502-025 | (2.5 U/μℓ) |
| | 500 U | 502-050 | |
| | 1,000 U | 502-100 | |
| Pfu DNA polymerase | 250 U | 503-025 | (2.5 U/μℓ) |
| | 500 U | 503-050 | |
| | 1,000 U | 503-100 | |

| Products | Scale | Size | Cat. No. | Type |
|---|---|---|---|---|

### GeneAll® *AmpONE™* *for PCR amplification*

| | | | | |
|---|---|---|---|---|
| Hotstart Taq DNA polymerase | | 250 U | 531-025 | (2.5 U/μℓ) |
| | | 500 U | 531-050 | |
| | | 1,000 U | 531-100 | |
| Clean Taq DNA polymerase | | 250 U | 551-025 | (2.5 U/μℓ) |
| | | 500 U | 551-050 | |
| | | 1,000 U | 551-100 | |
| Clean α-Taq DNA polymerase | | 250 U | 552-025 | (2.5 U/μℓ) |
| | | 500 U | 552-050 | |
| | | 1,000 U | 552-100 | |
| Taq Premix | 96 tubes | 20 μℓ | 521-200 | lyophilized |
| | | 50 μℓ | 521-500 | |
| | | 20 μℓ | 526-200 | solution |
| | | 50 μℓ | 526-500 | |
| α-Taq Premix | 96 tubes | 20 μℓ | 522-200 | lyophilized |
| | | 50 μℓ | 522-500 | |
| | | 20 μℓ | 527-200 | solution |
| | | 50 μℓ | 527-500 | |
| HS-Taq Premix | 96 tubes | 20 μℓ | 525-200 | solution |
| | | 50 μℓ | 525-500 | |
| Taq Premix (w/o dye) | 96 tubes | 20 μℓ | 524-200 | lyophilized |
| α-Taq Premix (w/o dye) | 96 tubes | 20 μℓ | 525-200 | solution |
| dNTP mix | | 500 μℓ | 509-020 | 2.5 mM each |
| dNTP set (set of dATP, dCTP, dGTP and dTTP) | | 1 ml x 4 tubes | 509-040 | 100 mM |

### GeneAll® *AmpMaster™* *for PCR amplification*

| | | | |
|---|---|---|---|
| Taq Master mix | 2x | 511-010 | 0.5 ml x 2 tubes |
| | 2x | 511-050 | 0.5 ml x 10 tubes |
| α-Taq Master mix | 2x | 512-010 | 0.5 ml x 2 tubes |
| | 2x | 512-050 | 0.5 ml x 10 tubes |
| HS-Taq Master mix | 2x | 545-010 | 0.5 ml x 2 tubes |
| | 2x | 545-050 | 0.5 ml x 10 tubes |

* Each dNTP is available

## Note .

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Do not hesitate to ask us any question.
We thank you for any comment or advice.

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