Ver 1.1

Ribospin™ Pathogen/TNA

DNA/RNA PURIFICATION HANDBOOK

GeneAll

Customer & Technical Support

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

We appreciate your comments and advice.

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This protocol handbook is included in :

GeneAll® Ribospin™ Pathogne/TNA (341-150, 341-152)

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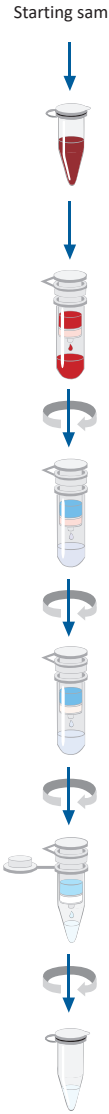
Brief protocol

Rapid protocol for fluid samples (serum/cell media/body fluid)



1. Transfer 200 μl of the sample to 1.5 ml microcentrifuge tube
2. Add 20 μl of Proteinase K solution and 200 μl of Buffer KL
3. Incubate for 5 min at 20~25°C

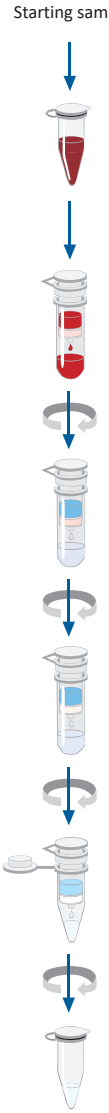
4. Add 300 μl of Buffer RB1
5. Transfer the mixture into mini column
6. Centrifuge for 1 min, ≥10,000 x g

7. Add 600 μl of Buffer RBW into mini column
8. Centrifuge for 1 min, ≥10,000 x g

9. Add 600 μl of Buffer RNW into mini column
10. Centrifuge for 1 min, ≥10,000 x g
11. Additional centrifuge for 1 min, full speed

12. Apply 50 μl of Nuclease-free water into mini column
13. Incubate for 1 min at RT
14. Centrifuge for 1 min, full speed

Standard protocol for fluid samples (serum/cell media/body fluid)



1. Transfer 200 μl of the sample to 1.5 ml microcentrifuge tube
2. Add 200 μl of Buffer SL
3. Add 20 μl of Proteinase K solution and 200 μl of Buffer KL
4. Incubate for 10 min at 20~25°C

5. Add 300 μl of Buffer RB1
6. Transfer the mixture into mini column
7. Centrifuge for 1 min, ≥10,000 x g
8. Repeat step 6~7

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

11. Add 600 μl of Buffer RNW into mini column
12. Centrifuge for 1 min, ≥10,000 x g
13. Additional Centrifuge for 1 min, full speed

14. Apply 50 μl of Nuclease-free water into mini column
15. Incubate for 1 min at RT
16. Centrifuge for 1 min, full speed

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Protocols

Kit Contents

Cat. No.	341-150	341-152	Storage
Type	mini		
Components	Quantity		
No. of preparation	50	250	Room temperature (15~25°C)
Column Type P (mini)	50	250	
1.5 ml microcentrifuge tube	50	250	
Buffer SL	34 ml	160 ml	
Buffer KL	20 ml	85 ml	
Buffer BL	15 ml	60 ml	
Buffer RB1 (concentrate) *	5 ml	17 ml	
Buffer RBW (concentrate) *	18 ml	77 ml	
Buffer RNW (concentrate) *	8 ml	34 ml	
Nuclease-free water	15 ml	90 ml	
PK Storage buffer	1.5 ml	7 ml	
Proteinase K **	24 mg	120 mg	
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** Before first use, add absolute ethanol (ACS grade or better) into Buffer RB1, RBW, RNW as indicated on the bottle.*

*** For the long-term storage of Proteinase K, store at 4°C. But after reconstitution of Proteinase K store at -20°C. Refer to instruction of Proteinase K on page 10.*

Materials Not Provide

- Reagent : Absolute ethanol (ACS grade or better)
  Powerbead™ tube (Protocol for Stool, Cat. No. 114-990 [50], 114-991 [6]).
  30 mg/ml lysozyme (LYS702, Bioshop, Canada, or equivalent)
  300 μg/ml lysostaphin (L7386, SIGMA, USA, or equivalent)
  Buffer GP (106-900~106-905, GeneAll, Korea)
- Disposable material : RNase-free pipette tips, Disposable gloves
- Equipment : Equipment for homogenizing sample, Microcentrifuge, Vortex mixer, Suitable protector

Product Specifications

Ribospin™ Pathogen/TNA	
Type	Spin
Maximum amount of starting samples	Liquid sample : 200 μl/prep Solid sample : 20 mg/prep Cultured cell : 5 x 10^6/prep
Preparation time	≥30 min
Maximum loading volume of mini column	750 μl
Minimum elution volume	30 μl

Quality Control

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

Storage Conditions

All components of GeneAll® Ribospin™ Pathogen/TNA should be stored at room temperature (15~25°C). It should be protected from exposure to direct sunlight. During shipment or storage under cool ambient condition, a precipitate can be formed in Buffer KL and BL. In such a case, heat the bottle to 56°C to dissolve completely. Using precipitated buffers will lead to poor DNA/RNA recovery. GeneAll® Ribospin™ Pathogen/TNA is guaranteed until the expiration date printed on the product box.

Safety Information

The buffers included in GeneAll® Ribospin™ Pathogen/TNA contain irritants which are harmful when in contact with skin or eyes, or when inhaled or swallowed. Care should be taken when handling such materials. Always wear gloves and eye protection, and follow standard safety precautions.
Buffer KL and BL contains chaotropic agents, which can form highly reactive compounds when combined with bleach.
Do NOT add bleach or acidic solutions directly to the sample-preparation waste.

Product Disclaimer

GeneAll® Ribospin™ Pathogen/TNA is for research use only, not for use in diagnostic procedure.

Preventing RNase Contamination

RNase can be introduced accidentally during RNA purification. Wear disposable gloves always, because skin often contains bacteria and molds that can be a source of RNase contamination. Use sterile, disposable plastic wares and automatic pipettes to prevent cross-contamination of RNase from shared equipment.

Proteinase K

This kit provides Proteinase K and PK Storage buffer for dissolving Proteinase K. Reconstituted Proteinase K serves efficient viral lysis for most sample types.
Proteinase K solution should be stored under 4°C for conservation of activity. It can be stored at 4°C for 1 year without significant decrease in activity.
To store for extended periods of time, it is recommended to store under -20°C.

Product Description

The GeneAll® Ribospin™ Pathogen/TNA kit provides a flexible format for the purification of the Pathogen and Total Nucleic Acids (TNA) from various samples such as serum, body fluid, tissues, whole blood, bacteria, swab, stool, raw milk and virus-infected samples.

The GeneAll® Ribospin™ Pathogen/TNA kit uses utilizes the advanced silica-binding technology to purify the total nucleic acids that become sufficiently pure for many applications. Viral samples are lysed in the optimized buffer comprising detergent and lytic enzyme. Under optimized binding conditions, nucleic acids in the lysate bind to silica membrane and impurities pass through the membranes into a collection tube.

The membranes are washed with a series of alcohol-containing buffers to remove traces of proteins, cellular debris and salts.

Finally, pure nucleic acids are released into a clean microcentrifuge tube with deionized water or low ionic strength buffer. The elute should be treated carefully because RNA are very sensitive to contaminants such as RNases, often found on general labware and dust.

Purified nucleic acids can be used directly for PCR, qPCR, RT-PCR, or any downstream application without further manipulation.

Sample amount and expected yield

Sample	Starting amount	Yield (μg)
Cultured cell	5 x 10^6	60~90
Whole blood (Human)	200 μl	2~6
Whole blood (Pig)	200 μl	10~20
Heart	20 mg	20~50
Lung	20 mg	80~120
Spleen	10 mg	100~130
Stomach	20 mg	60~100
Intestein	20 mg	100~130
Liver	20 mg	50~80
Kidney	20 mg	60~100
Brain	20 mg	20~50
Stool (Pig)	50 mg	10~30
Raw milk	1 ml	2~4

A. PROTOCOL FOR Body fluid/Cultured cells (Rapid protocol)

Before experiment

- *Before using for the first time, add absolute ethanol (ACS grade or better). into Buffer RB1, RBW and RNW as indicated on the bottle.*
- *Prepare 1.5 ml microcentrifuge tube.*
- *All centrifugation should be performed at room temperature.*
- *Prepare Proteinase K solution (20 mg/ml) for first use.*
- *If a precipitate has formed in Buffer KL, heat to dissolve at 56°C before use.*

1. **Transfer 200 µl of sample to the 1.5 ml microcentrifuge tube. Use the starting sample listed below.**
   If the sample volume is less than 200 µl, adjust the volume to 200 µl with 1X PBS.

Sample	Max. amount per prep	Preparation
Cultured cells or lymphocyte	5 x 10^6 cells	5 x 10^6 cells in 200 µl of 1X PBS
Body fluid	200 µl	Direct use
Serum	200 µl	Direct use
Virus in culture median	200 µl	200 µl of virus-containing media

2. **Add 20 µl of Proteinase K solution (20 mg/ml, provided) and 200 µl of Buffer KL to the sample. Vortex vigorously to mix thoroughly.**

3. **Incubate at 20~25°C for 5 min.**
   Changing the incubation temperature to 56°C may increase the extraction efficiency of bacteria, but it is not recommended as the changed condition of lysis could not preserve the RNA integrity.

4. **Add 300 µl of Buffer RB1 to the sample, pulse-vortex to mix the sample thoroughly, and spin down briefly to remove any drops from inside of the lid.**

5. **Transfer the mixture to the Column Type P (mini) carefully, centrifuge at 10,000 x g above for 1 min, and discard the pass-through and reinsert the mini column back into the collection tube.**

6. **Add 600 µl of Buffer RBW to the mini column, centrifuge at 10,000 x g above for 1 min, and discard the pass-through and reinsert the mini column back into the collection tube.**

7. **Add 600 µl of Buffer RNW to the mini column, centrifuge at 10,000 x g above for 1 min, and discard the pass-through and reinsert the mini column back into the collection tube.**

8. **Centrifuge at full speed for 1 min and remove residual wash buffer.**
   Place the mini column into a fresh 1.5 ml microcentrifuge tube.

9. **Add 50 µl of nuclease-free water to the center of the membrane in the mini column.**
   Incubate at room temperature for 1 min.

10. **Centrifuge at full speed for 1 min.**

B. PROTOCOL FOR Body fluid/Cultured cells (Standard protocol)

Before experiment

- *Before using for the first time, add absolute ethanol (ACS grade or better). into Buffer RB1, RBW and RNW as indicated on the bottle.*
- *Prepare 1.5 ml microcentrifuge tube.*
- *All centrifugation should be performed at room temperature.*
- *Prepare Proteinase K solution (20 mg/ml) for first use.*
- *If a precipitate has formed in Buffer KL, heat to dissolve at 56°C before use.*

1. **Transfer 200 µl of sample to the 1.5 ml microcentrifuge tube. Use the starting sample listed below.**
   If the sample volume is less than 200 µl, adjust the volume to 200 µl with 1X PBS.

Sample	Max. amount per prep	Preparation
Cultured cells or lymphocyte	5 x 10^6 cells	5 x 10^6 cells in 200 µl of 1X PBS
Body fluid	200 µl	Direct use
Serum	200 µl	Direct use
Virus in culture median	200 µl	200 µl of virus-containing media
Gram-negative bacteria	Up to 2 x 10^9 cells	2 x 10^9 cells in 200 µl of 1X PBS

2. **Add 200 µl of Buffer SL to the sample and vortex to mix thoroughly.**

3. **Add 20 µl of Proteinase K solution (20 mg/ml, provided) and 200 µl of Buffer KL to the sample. Vortex vigorously to mix thoroughly.**

4. **Incubate at 20~25°C for 10 min.**
   Changing the incubation temperature to 56°C may increase the bacteria DNA recovery, but it is not recommended as the changed condition of lysis could not preserve the RNA integrity.

5. **Add 300 µl of Buffer RB1 to the sample, pulse-vortex to mix the sample thoroughly, and spin down briefly to remove any drops from inside of the lid.**

6. **Transfer the mixture to the Column Type P (mini) carefully, centrifuge at 10,000 x g above for 1 min, and discard the pass-through and reinsert the mini column back into the collection tube.**

7. **Repeat step 6 with the remainder of the sample.**

8. **Add 600 µl of Buffer RBW to the mini column, centrifuge at 10,000 x g above for 1 min, and discard the pass-through and reinsert the mini column back into the collection tube.**

9. **Add 600 µl of Buffer RNW to the mini column, centrifuge at 10,000 x g above for 1 min, and discard the pass-through and reinsert the mini column back into the collection tube.**

10. **Centrifuge at full speed for 1 min and remove residual wash buffer. Place the mini column into a fresh 1.5 ml microcentrifuge tube.**

11. **Add 50 µl of nuclease-free water to the center of the membrane in the mini column.**
    Incubate at room temperature for 1 min.

12. **Centrifuge at full speed for 1 min.**

C. PROTOCOL FOR Whole blood

Before experiment

- *Before using for the first time, add absolute ethanol (ACS grade or better). into Buffer RB1, RBW and RNW as indicated on the bottle.*
- *Prepare 1.5 ml microcentrifuge tube.*
- *All centrifugation should be performed at room temperature.*
- *Prepare Proteinase K solution (20 mg/ml) for first use.*
- *If a precipitate has formed in Buffer BL, heat to dissolve at 56°C before use.*

1. **Transfer 200 µl of whole blood to the 1.5 ml microcentrifuge tube.**

2. **Add 200 µl of Buffer SL to the sample and vortex to mix thoroughly.**

3. **Add 20 µl of Proteinase K solution (20 mg/ml, provided) and 200 µl of Buffer BL to the sample. Vortex vigorously to mix thoroughly.**
   Use Buffer BL instead of Buffer KL in the case of blood samples. The Buffer KL can cause blood clotting.

4. **Incubate at 20~25°C for 10 min.**
   Changing the incubation temperature to 56°C may increase bacteria DNA recovery, but it is not recommended as changed condition of lysis could not preserve RNA integrity.

5. **Add 300 µl of Buffer RB1 to the sample, pulse-vortex to mix the sample thoroughly, and spin down briefly to remove any drops from inside of the lid.**

6. **Transfer the mixture to the Column Type P (mini) carefully, centrifuge at 10,000 x g above for 1 min, and discard the pass-through and reinsert the mini column back into the collection tube.**

7. **Repeat step 6 with the remainder of the sample.**

8. **Add 600 µl of Buffer RBW to the mini column, centrifuge at 10,000 x g above for 1 min, and discard the pass-through and reinsert the mini column back into the collection tube.**

9. **Add 600 µl of Buffer RNW to the mini column, centrifuge at 10,000 x g above for 1 min, and discard the pass-through and reinsert the mini column back into the collection tube.**

10. **Centrifuge at full speed for 1 min and remove residual wash buffer. Place the mini column into a fresh 1.5 ml microcentrifuge tube.**

11. **Add 50 µl of nuclease-free water to the center of the membrane in the mini column.**
    Incubate at room temperature for 1 min.

12. **Centrifuge at full speed for 1 min.**

D. PROTOCOL FOR Tissue

Before experiment

- *Before using for the first time, add absolute ethanol (ACS grade or better). into Buffer RB1, RBW and RNW as indicated on the bottle.*
- *Prepare 1.5 ml microcentrifuge tube.*
- *All centrifugation should be performed at room temperature.*
- *Prepare Proteinase K solution (20 mg/ml) for first use.*
- *If a precipitate has formed in Buffer KL, heat to dissolve at 56°C before use.*

1. **Homogenize up to 10~20 mg of tissue as described in step 1-1, 1-2 or 1-3, depending on the sample type.**
   Homogenizing the sample finely will accelerate lysis and decrease the lysis time.
   For spleen or stomach tissue, up to 10 mg can be processed.

   ◆ **1-1 For soft tissue, such as liver or brain, put up to 20 mg of the tissue into 1.5 ml microcentrifuge tube (not provided), add 300 µl of Buffer SL, and homogenize thoroughly with microhomogenizer.**

   ◆ **1-2 If microhomogenizer is not available or the tissue is not soft, grind the tissue to a fine powder with liquid nitrogen in a pre-chilled mortar and pestle. Put up to 20 mg of the powdered tissue into 1.5 ml microcentrifuge tube. Add 300 µl of Buffer SL and pulse-vortex for 15 sec.**

   ◆ **1-3 If neither 1a nor 1b is available, mince the tissue with sharp blade or scalpel as small as possible. Put the tissue into a 1.5 ml microcentrifuge tube.**
   Add 300 µl of Buffer SL and pulse-vortex for 15 sec.

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

2. **Incubate at room temperature for 5 min.**

3. ***(Optional:)*** **If many air bubbles form from the samples, spin down briefly within 20 sec at 13,000 rpm to remove the bubbles and not the homogenized tissue piece.**
   Do not exceed 20 sec to increase extraction efficiency of bacteria, exceed 20 sec of centrifugation lead to sink bacteria.

4. **Transfer 200 μl of the lysate except the bubbles and piece of tissues to the new 1.5 ml microcentrifuge tube.**

5. **Add 20 μl of Proteinase K solution (20 mg/ml, provided) and 200 μl of Buffer KL and to the tube. Vortex vigorously to mix thoroughly.**

6. **Incubate at room temperature for 10 min.**

7. **Add 300 μl of Buffer RB1 to the sample, pulse-vortex to mix the sample thoroughly, and spin down briefly to remove any drops from inside of the lid.**

8. **Transfer the mixture to the Column Type P (mini) carefully, centrifuge at 10,000 x g above for 1 min, and discard the pass-through and reinsert the mini column back into the collection tube.**

9. **Add 600 μl of Buffer RBW to the mini column, centrifuge at 10,000 x g above for 1 min, and discard the pass-through and reinsert the mini column back into the collection tube.**

10. **Add 600 μl of Buffer RNW to the mini column, centrifuge at 10,000 x g above for 1 min, and discard the pass-through and reinsert the mini column back into the collection tube.**

11. **Centrifuge at full speed for 1 min and remove residual wash buffer. Place the mini column into a fresh 1.5 ml microcentrifuge tube.**

12. **Add 50~200 μl of nuclease-free water to the center of the membrane in the mini column. Incubate at room temperature for 1 min.**

13. **Centrifuge at full speed for 1 min.**

14. **Dilute the elute to 20~250 ng/μl to use the template for RT-PCR or PCR.**
    Tissue samples usually have many cells. The total nucleic acid of cells from animal tissue can be competitors with the nucleic acid of the pathogen in PCR reactions.

E. PROTOCOL FOR Stool/Fecal swab

Before experiment

- *Before using for the first time, add absolute ethanol (ACS grade or better). into Buffer RB1, RBW and RNW as indicated on the bottle.*
- *Prepare 1.5 ml microcentrifuge tube.*
- *All centrifugation should be performed at room temperature.*
- *Prepare Proteinase K solution (20 mg/ml) for first use.*
- *If a precipitate has formed in Buffer KL, heat to dissolve at 56°C before use.*

1. Collect the samples as described in step 1-1, 1-2 depending on the sample type.

◆ **1-1 Stool**

: Add up to 50 mg of stool sample to a 1.5 ml microcentrifuge tube.

To increase the extraction efficiency and purity, using a Powerbead™ tube instead of a 1.5 ml micocentrifuge tube is recommended. The Powerbead™ tube is not provided in this kit. The Powerbead™ tube can be purchased separately (Cat. No. 114-990 [50], 114-991 [6]).

◆ **1-2 Fecal swab**

: Vortex fecal swab tube vigorously to mix thoroughly and transfer 200 µl of the sample to a 1.5 ml microcentrifuge tube.

2. Add 600 µl of Buffer SL to the tube and vortex for 2 min or until the stool sample is thoroughly homogenized.

It is important to homogenize the sample thoroughly. Insufficient homogenization time and condition is related to low recovery yield.

3. Centrifuge the samples as described in step 3-1, 3-2 depending on the pathogen type.

◆ **3-1 <u>For extraction of virus</u>;**
Centrifuge at 13,000 rpm for 5 min at room temperature and carefully transfer the 300 µl of supernatant to a 1.5 ml microcentrifuge tube.

◆ **3-2 <u>For extraction of bacteria and nucleic acid of stool</u>;**
Centrifuge at 1,000 rpm for 30 sec at room temperature and carefully transfer the 300 µl of supernatant to a 1.5 ml microcentrifuge tube.
Centrifugation at 1,000 rpm for 30 sec is for the sinking debris of stool.
Do not exceed 30 sec to increase the extraction efficiency of bacteria, exceeding 30 sec of centrifugation can lead to sink bacteria.

4. **Add 20 µl of Proteinase K solution (20 mg/ml, provided) and 300 µl of Buffer KL to the sample. Vortex vigorously to mix thoroughly.**

5. **Incubate at 20~25°C for 5 min.**

6. **Add 300 µl of Buffer RB1 to the sample, pulse-vortex to mix the sample thoroughly, and spin down briefly to remove any drops from inside of the lid.**

7. **Transfer the mixture to the Column Type P (mini) carefully, centrifuge at 6,000 x g above (>8,000 rpm) for 1 min, and discard the pass-through and reinsert the mini column back into the collection tube.**

8. **Repeat step 7 with the remainder of the sample.**

9. **Add 600 µl of Buffer RBW to the mini column, centrifuge at 10,000 x g above for 1 min, and discard the pass-through and reinsert the mini column back into the collection tube.**

10. **Add 600 µl of Buffer RNW to the mini column, centrifuge at 10,000 x g above for 1 min, and discard the pass-through and reinsert the mini column back into the collection tube.**

11. **Centrifuge at full speed for 1 min and remove residual wash buffer. Place the mini column into a fresh 1.5 ml microcentrifuge tube.**

12. **Add 50~200 µl of nuclease-free water to the center of the membrane in the mini column. Incubate at room temperature for 1 min.**
PCR inhibitor in samples such as stool can obstruct PCR reaction. Dilute the elute to use the template for PCR reactions.

13. **Centrifuge at full speed for 1 min.**

F. PROTOCOL FOR Saliva, Nasopharyngeal aspirates, Buccal swabs

Before experiment

- *Before using for the first time, add absolute ethanol (ACS grade or better). into Buffer RB1, RBW and RNW as indicated on the bottle.*
- *Prepare 1.5 ml microcentrifuge tube.*
- *All centrifugation should be performed at room temperature.*
- *Prepare Proteinase K solution (20 mg/ml) for first use.*
- *If a precipitate has formed in Buffer KL, heat to dissolve at 56°C before use.*

1. Collect the samples as described in step 1-1, 1-2 depending on the sample type.

◆ **1-1 Saliva**

: 400 µl of saliva in a 1.5 ml microcentrifuge tube. add 600 µl of 1X PBS to the sample and vortex until the saliva sample is thoroughly homogenized.

Collect saliva on ice and as quickly as possible or use RNA protect saliva reagent. Because the RNA in saliva is unstable and easy to degrade.

◆ **1-2 Nasopharyngeal aspirates**

: Collect 1 ml of nasopharyngeal aspirates in a 1.5 ml microcentrifuge tube and vortex until the sample is thoroughly homogenized.

◆ **1-3 Buccal swab**

: Vortex buccal swab tube vigorously to mix thoroughly and transfer 300 µl of the sample to a 1.5 ml microcentrifuge tube.

2. Centrifuge the samples as described in step 2-1, 2-2 depending on the pathogen type.

◆**2-1 <u>For extraction of virus</u>;**

1) Centrifuge at 13,000 rpm for 5 min at room temperature and carefully transfer the 200 μl of supernatant to a 1.5 ml microcentrifuge tube.

2) Add 200 μl of Buffer SL to the sample and vortex vigorously to mix.

◆**2-2 <u>For extraction of bacteria and nucleic acid of saliva</u>;**

1) Centrifuge at 13,000 rpm for 5 min at room temperature and carefully discard the supernatant.

2) Add 200 μl of Buffer SL to the pellet and resuspend completely the pellets in Buffer SL.

3. **Add 20 μl of Proteinase K solution (20 mg/ml, provided) and 300 μl of Buffer KL to the sample. Vortex vigorously to mix thoroughly.**

4. **Incubate at 20~25°C for 5 min.**

5. **Add 300 μl of Buffer RB1 to the sample, pulse-vortex to mix the sample thoroughly, and spin down briefly to remove any drops from inside of the lid.**

6. **Transfer the mixture to the Column Type P (mini) carefully, centrifuge at 10,000 x g above for 1 min, and discard the pass-through and reinsert the mini column back into the collection tube.**

7. **Repeat step 6 with the remainder of the sample.**

8. **Add 600 μl of Buffer RBW to the mini column, centrifuge at 10,000 x g above for 1 min, and discard the pass-through and reinsert the mini column back into the collection tube.**

9. **Add 600 μl of Buffer RNW to the mini column, centrifuge at 10,000 x g above for 1 min, and discard the pass-through and reinsert the mini column back into the collection tube.**

10. **Centrifuge at full speed for 1 min and remove residual wash buffer. Place the mini column into a fresh 1.5 ml microcentrifuge tube.**

11. **Add 50 μl of nuclease-free water to the center of the membrane in the mini column. Incubate at room temperature for 1 min.**

12. **Centrifuge at full speed for 1 min.**

G. PROTOCOL FOR
Raw milk

This pretreatment is for the extraction of the bacteria and the nucleic acid of raw milk. It is not suitable for virus because of the centrifugation step.

Before experiment

- *Before using for the first time, add absolute ethanol (ACS grade or better). into Buffer RB1, RBW and RNW as indicated on the bottle.*
- *Prepare 1.5 ml microcentrifuge tube.*
- *All centrifugation should be performed at room temperature.*
- *Prepare Proteinase K solution (20 mg/ml) for first use.*
- *If a precipitate has formed in Buffer KL, heat to dissolve at 56°C before use.*

1. **Transfer 1 ml of raw milk to the 1.5 ml microcentrifuge tube.**
2. **Centrifuge at 10,000 x g above for 5 min at room temperature and discard the supernatant containing fat and liquid layer.**
3. **Add 200 µl of Buffer SL to the pellet and resuspend completely the pellets in Buffer SL.**
4. **Add 20 µl of Proteinase K solution (20 mg/ml, provided) and 200 µl of Buffer KL to the sample. Vortex vigorously to mix thoroughly.**
5. **Incubate at 20~25°C for 5 min.**
6. **Add 300 µl of Buffer RB1 to the sample, pulse-vortex to mix the sample thoroughly, and spin down briefly to remove any drops from inside of the lid.**
7. **Transfer the mixture to the Column Type P (mini) carefully, centrifuge at 10,000 x g above for 1 min, and discard the pass-through and reinsert the mini column back into the collection tube.**

8. **Add 600 µl of Buffer RBW to the mini column, centrifuge at 10,000 x g above for 1 min, and discard the pass-through and reinsert the mini column back into the collection tube.**

9. **Add 600 µl of Buffer RNW to the mini column, centrifuge at 10,000 x g above for 1 min, and discard the pass-through and reinsert the mini column back into the collection tube.**

10. **Centrifuge at full speed for 1 min and remove residual wash buffer. Place the mini column into a fresh 1.5 ml microcentrifuge tube.**

11. **Add 50 µl of nuclease-free water to the center of the membrane in the mini column. Incubate at room temperature for 1 min.**

12. **Centrifuge at full speed for 1 min.**

H. PROTOCOL FOR Dried blood spot

Before experiment

- *Before using for the first time, add absolute ethanol (ACS grade or better). into Buffer RB1, RBW and RNW as indicated on the bottle.*
- *Prepare 1.5 ml microcentrifuge tube.*
- *All centrifugation should be performed at room temperature.*
- *Prepare Proteinase K solution (20 mg/ml) for first use.*
- *If a precipitate has formed in Buffer BL, heat to dissolve at 56°C before use.*

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

1. **Place 3~4 punched-out circles from a dried blood spot into a 1.5 ml microcentrifuge tube and add 200 µl of Buffer SL.**
   Use a 3 mm (1/8") single-hole paper puncher to cut out the circles from a dried blood spot.

2. **Incubate at 56°C for 10 min. Spin down briefly to remove any drops from inside of the lid.**
   Do not incubate for more than 15 min.

3. **Add 20 µl of Proteinase K solution (20 mg/ml, provided) and 200 µl of Buffer BL to the sample. Vortex vigorously to mix thoroughly and spin down briefly to remove any drops from inside of lid.**
   Use Buffer BL instead of Buffer KL in the case of blood samples. The Buffer KL can cause blood clotting.

4. **Incubate at 56°C for 10 min.**

5. **Transfer the lysate except paper to the 1.5 ml mi microcentrifuge tube.**

6. **Add 300 µl of Buffer RB1 to the sample, pulse-vortex to mix the sample thoroughly, and spin down briefly to remove any drops from inside of the lid.**

7. **Transfer the mixture to the Column Type P (mini) carefully, centrifuge at 10,000 x g above for 1 min, and discard the pass-through and reinsert the mini column back into the collection tube.**

8. **Add 600 µl of Buffer RBW to the mini column, centrifuge at 10,000 x g above for 1 min, and discard the pass-through and reinsert the mini column back into the collection tube.**

9. **Add 600 µl of Buffer RNW to the mini column, centrifuge at 10,000 x g above for 1 min, and discard the pass-through and reinsert the mini column back into the collection tube.**

10. **Centrifuge at full speed for 1 min and remove residual wash buffer.**
    Place the mini column into a fresh 1.5 ml microcentrifuge tube.

11. **Add 50 µl of nuclease-free water to the center of the membrane in the mini column.**
    Incubate at room temperature for 1 min.

12. **Centrifuge at full speed for 1 min.**

I. PROTOCOL FOR Gram positive bacteria

Before experiment

- *Before using for the first time, add absolute ethanol (ACS grade or better). into Buffer RB1, RBW and RNW as indicated on the bottle.*
- *Prepare 1.5 ml microcentrifuge tube.*
- *All centrifugation should be performed at room temperature.*
- *Prepare Proteinase K solution (20 mg/ml) for first use.*
- *If a precipitate has formed in Buffer KL, heat to dissolve at 56°C before use.*

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

  30 mg/ml lysozyme (LYS702, Bioshop, Canada, or equivalent)
  or/and
  300 μg/ml lysostaphin (L7386, SIGMA, USA, or equivalent)
  Buffer GP (106-900~106-905, GeneAll, Korea)

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

1. **Harvest cells (up to 2 x 10^9 cells) in a 1.5 ml microcentrifuge tube by centrifugation at full speed for 1 min. Discard the supernatant.**

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

3. **Add 200 µl of Buffer SL to the sample. Vortex to mix thoroughly.**

4. **Add 20 µl of Proteinase K solution (20 mg/ml, provided) and 200 µl of Buffer KL to the sample. Vortex vigorously to mix thoroughly.**

5. **Incubate at 20~25˚C for 5 min.**

6. **Add 300 µl of Buffer RB1 to the sample, pulse-vortex to mix the sample thoroughly, and spin down briefly to remove any drops from inside of the lid.**

7. **Transfer the mixture to the Column Type P (mini) carefully, centrifuge at 10,000 x g above for 1 min, and discard the pass-through and reinsert the mini column back into the collection tube.**

8. **Repeat step 7 with the remainder of the sample.**

9. **Add 600 µl of Buffer RBW to the mini column, centrifuge at 10,000 x g above for 1 min, and discard the pass-through and reinsert the mini column back into the collection tube.**

10. **Add 600 µl of Buffer RNW to the mini column, centrifuge at 10,000 x g above for 1 min, and discard the pass-through and reinsert the mini column back into the collection tube.**

11. **Centrifuge at full speed for 1 min and remove residual wash buffer. Place the mini column into a fresh 1.5 ml microcentrifuge tube.**

12. **Add 50 µl of nuclease-free water to the center of the membrane in the mini column. Incubate at room temperature for 1 min.**

13. **Centrifuge at full speed for 1 min.**

Troubleshooting Guide

Facts	Possible Causes	Suggestions
Low yield	Poor quality of starting material	Too old or improperly stored sample often yield degraded DNA/RNA. Use fresh sample, if possible. Repeated freezing and thawing the sample should be avoided.
	Low cells in the sample	Some samples such as serum/plasma may have low concentration of cells. To increase the binding efficiency of the nucleic acid, it is recommended to use Carrier RNA. Carrier RNA can be purchased separately (Cat. No. 118-962 [270 μg], 118~963 [370 μg]).
	Inefficient or insufficient lysis	For proper lysis, the complete mix of sample and Buffer KL is essential.
	Improper elution	Add Nuclease-free water to the center of the mini column membrane and perform incubation for 1 min before centrifugation.
	Precipitation of Buffer KL and BL	Storage at cool ambient temperature may cause precipitation in Buffer KL and BL. For a good result, any precipitate in the buffer should be dissolved by heating the buffer at 37°C or above until it disappears.
	Degradation of RNA	RNase can be introduced during purification of nucleic acid. Be certain not to introduce any RNases during the procedure or later handling. Keep tubes closed whenever possible during the extraction and use RNase-free products with sterile and disposable plastic ware.
	Column clogging	Reduce the amount of starting sample. Especially tissue sample, use the correct amount of starting sample.

Facts	Possible Causes	Suggestions
	PCR inhibitors present in samples	PCR inhibitor in samples such as stool can obstruct PCR reaction. Dilute the elute to use the template for PCR reactions.
	Too high concentration of cells in animal tissues	Tissue samples usually have many cells. The total nucleic acid of cells from animal tissue can be competitors with the nucleic acid of the pathogen in PCR reactions. Dilute the elute to 20~250 ng to use the template for PCR reactions.
Eluate does not perform well In downstream Application	**Buffer RB1, RBW, or RNW was prepared incorrectly**	Check that the concentrated Buffer RB1, RBW, and RNW were diluted with the correct volume of absolute ethanol.
	Residual ethanol from Buffer RNW remains in elute	Care must be taken for eliminating the carryover of Buffer RNW before elution step. The membrane of mini spin column should be kept completely dry via additional centrifugation or air-drying.
	Use of Buffer RBW and RNW in the wrong order	Ensure that Buffer RBW and RNW are used in the correct order in the protocol. If used in the wrong order, perform the last washing step with RNW.

APPENDIX I

DNase I treatment in eluate

Treatment with DNase I is an optional step to eliminate DNA in eluate depending on purpose of experiment.
Appendix I describes how to use the DNase I (**Not** included in this kit).
This procedure is more efficient than on-column DNase I treatment.

Protocol

1. **Prepare the mixture as below in a 1.5 ml microcentrifuge tube.**
   - 50 μl eluate
   - 5 μl Buffer DRB
   - 1 μl DNase I solution (Cat. No. 307-928)

2. **Incubate the mixture for 10 min at room temperature.**

3. **Add 1 μl 0.25 M EDTA per 50 μl eluate.**

4. **Inactivate DNase I enzyme at 75°C for 10 min.**

** For efficient DNase I treatment and clean-up of eluated RNA, use of Riboclear™ plus (Cat.No. 313-150) is suggested.*

Related product

Product	Cat. No.	Size	Features and Benefits
Riboclear™ plus	313-150	50 prep	- Preparation time : ~17 min - High recovery rate : ~95% - Efficient removal of genomic DNA including DNase I - Stable and consistent yield - Concentrated RNA eluate using micro column - Complete removal of salt and enzymes - No use of organic solvents, no ethanol precipitation

APPENDIX II

RNase A treatment in eluate

Treatment with RNase A is an optional step to eliminate RNA in eluate depending on purpose of experiment.
Appendix II describes how to use the RNase A (**Not** included in this kit).

Protocol

1. **Prepare the mixture as below in a 1.5 ml microcentrifuge tube.**
   - 50 μl eluate
   - 4 μl RNase solution (100 mg/ml, Cat. No. 117-960)

2. **Vortex to mix thoroughly.**

3. **Incubate the mixture for 10 min at 37°C.**

Ordering Information

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Products	Scale	Size	Cat. No.	Type

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

GenEx™ Plant	Sx	100	227-101	solution
	Mx	100	227-201	
	Lx	100	227-301	
GenEx™ Plant *plus!*	Sx	100	228-101	solution
	Mx	50	228-250	
	Lx	20	228-320	

GeneAll® *DirEx™* series

for preperation of PCR-template without extraction

DirEx™		100	250-101	solution
DirEx™ *Fast*-Tissue		96 T	260-011	solution
DirEx™ *Fast*-Cultured cell		96 T	260-021	solution
DirEx™ *Fast*-Whole blood		96 T	260-031	solution
DirEx™ *Fast*-Blood stain		96 T	260-041	solution
DirEx™ *Fast*-Hair		96 T	260-051	solution
DirEx™ *Fast*-Buccal swab		96 T	260-061	solution
DirEx™ *Fast*-Cigarette		96 T	260-071	solution

GeneAll® RNA series *for preperation of total RNA*

RiboEx™	mini	100	301-001	solution
		200	301-002	
Hybrid-R™	mini	100	305-101	spin
Hybrid-R™ Blood RNA	mini	50	315-150	spin
Hybrid-R™ miRNA	mini	50	325-150	spin
RiboEx™ LS	mini	100	302-001	solution
		200	302-002	
Riboclear™	mini	50	303-150	spin
Riboclear™ *plus!*	mini	50	313-150	spin
Ribospin™	mini	50	304-150	spin
Ribospin™ II	mini	50	314-150	spin
		300	314-103	
Ribospin ™ vRD	mini	50	302-150	spin
Ribospin ™ vRD *plus!*	mini	50	312-150	spin
Ribospin ™ vRD II	mini	50	322-150	spin
Ribospin ™ Plant	mini	50	307-150	spin
Ribospin™ Seed / Fruit	mini	50	317-150	spin
Allspin™	mini	50	306-150	spin
RiboSaver™	mini	100	351-001	solution

Products	Scale	Size	Cat. No.	Type

GeneAll® *AmpONE™* *for PCR amplification*

Taq DNA polymerase		250 U	501-025	(2.5 U/μl)
		500 U	501-050	
		1,000 U	501-100	
Taq Premix		20 μl x 96 tubes	526-200	solution
		50 μl x 96 tubes	526-500	

GeneAll® *AmpMaster™* *for PCR amplification*

Taq Master mix	0.5 ml x 2 tubes	541-010	solution
	0.5 ml x 10 tubes	541-050	solution

GeneAll® *HyperScript™* *for Reverse Transcription*

Reverse Transcriptase	10,000 U	601-100	solution
RT Master mix	0.5 ml x 2 tubes	601-710	solution
One-step RT-PCR Master mix	0.5 ml x 2 tubes	602-110	solution
One-step RT-PCR Premix	20 μl x 96 tubes	602-102	solution

GeneAll® RealAmp™ *for qPCR amplification*

SYBR qPCR Master mix (2X, Low ROX)	200 rxn	2 ml	801-020	solution
	500 rxn	5 ml	801-050	
SYBR qPCR Master mix (2X, High ROX)	200 rxn	2 ml	801-021	solution
	500 rxn	5 ml	801-051	

Products	Scale	Size	Cat. No.	Type

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™ 32 *Ultimately flexible automatic extraction system*

Automatic extraction equipment		GTI032	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

GeneAll® GENTi™ 32 ADVANCED *Ultimately flexible automatic extraction system*

Automatic extraction equipment		GTI032A	system
Genomic DNA	48	901-048A	tube
	96	901-096A	plate
Viral DNA / RNA	48	902-048A	tube
	96	902-096A	plate
Plant DNA / RNA	48	904-048A	tube
	96	904-096A	plate

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GeneAll

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