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PrimeWay Genomic DNA Extraction Kit (KIT-9020)

Sample Types

- Human/ Animal Tissue
- Cultured Cells
- Mouse/ Rat Tails
- Bacteria
- Yeast/ Fungi
- Blood on FTA Card
- Blood
- Buffy Coat
- Insects
- Buccal Swabs

Molecular Biology Kit

PrimeWay Genomic DNA Extraction Kit

Product No: KIT-9020

PrimeWay Genomic DNA Extraction Kit is a rapid and reliable kit that isolates highly pure genomic DNA from human/ animal tissue, cultured cells, mouse/ rat tails, bacteria, yeast/ fungi, dried blood spots, blood, buffy coat, insects as well as from buccal swabs. It uses a silica-based spin column and is suitable to isolate and purify DNA with approximate 20 minutes/prep. This handling time is yet to include the lysis steps, which the duration is vary according to sample type. The quality of the purified DNA is suitable for PCR, restriction analysis and Southern blotting etc. The extraction protocol for buffy coat is suitable for Next-Generation Sequencing.

For Research Use Only. Not for use in Diagnostic Procedures.

Kit Contents

No	Product	KIT-9020-10 10 preps	KIT-9020-50 50 preps	KIT-9020-250 250 preps
1	TLB1 Buffer	4 mL	20 mL	100 mL
2	TLB2 Buffer	3 mL	15 mL	75 mL
3	Wash Buffer T1	6 mL	30 mL	150 mL
4	Wash Buffer T2	2 mL	12 mL	50 mL
5	Elution Buffer	1.5 mL	13 mL	60 mL
6	Proteinase K	6 mg	30 mg	2 x 75 mg
7	Proteinase Buffer	1.8 mL	1.8 mL	8 mL
8	PrimeWay Genomic Column	10 pcs	50 pcs	5 x 50 pcs
9	Collection Tube	2 x 10 pcs	2 x 50 pcs	10 x 50 pcs

Storage

Store this kit at room temperature (21 – 25 °C). After reconstituted the Proteinase K, store at -20 °C and stable for at least 6 months.

Product Specification

	KIT-9020
Binding capacity	60 µg
Yield	20 – 35 µg
Sample Size	Refer **Table A**
Elution	60 – 100 µL
Handling	~20 minutes/prep (sample type dependent)

***Table A**: Each protocol is optimized with the listed sample size according to different sample type.*

Protocol	Sample Type	Sample Size	Page
A	Human/ Animal Tissue	≤ 25 mg	6 – 7
B	Cultured Cells	$10^2 – 10^7$	8 – 9
C	Mouse/ Rat Tails	≤ 2 pcs (0.6 cm each)	10 – 11
D	Bacteria	≤ 1 mL culture/ 20 mg cell pellet	12 – 14
E	Yeast/ Fungi	3 mL YPD yeast culture/ 30 mg cell pellet	15 – 17
F	Blood on FTA Card	≤ 2 spots (15 – 30 mm^2 each)	18 – 19
G	Blood	≤ 200 µL	20 – 21
H	Buffy coat	~250 µL	22 – 23
I	Insects	≤ 50 mg	24 – 25
J	Buccal Swabs	1 piece	26 - 27

Material Supplied by Users

- ✓ Ethanol (96% - 100%)
- ✓ Water bath or thermal block to set up to 56 °C
- ✓ Vortex mixer
- ✓ Centrifuge, at speed of 11,000 ×g
- ✓ Microcentrifuge tubes (1.5 mL or 2.0 mL respectively)
- ✓ Pipettes & pipette tips
- ✓ Refer **Table B** for the additional reagents may be required for different sample type respectively

***Table B**: For product ordering information, please refer the last page of this manual.*

Protocol	Sample Type	Reagents
A	Human/ Animal Tissue	✓ RNase A solution, 10 mg/mL
D	Bacteria	✓ Bacteria Pre-Lysis Buffer ✓ Lysozyme ✓ RNase A solution, 10 mg/mL
E	Yeast/ Fungi	✓ 10 mM EDTA, pH 8.0 ✓ Sorbitol Buffer *[1.2 M Sorbitol; 10 mM CaCl2; 0.1 M Tris-HCl pH 7.5; 35 mM β-mercaptoethanol]* ✓ Zymolyase ✓ RNase A solution, 10 mg/mL
H	Buffy Coat	✓ RNase A solution, 10 mg/mL ✓ Chloroform
I	Insects	✓ RNase A solution, 10 mg/mL
J	Buccal Swabs	✓ 1X Phosphate Buffered Saline (PBS)

Precautions for Users

- ✓ Some buffer of this kit contains irritants. Handle with care and avoid contact with skin. In case of contact, wash skin with a copious amount of water; seek medical attention.
- ✓ Always wear a lab coat, disposable gloves, and surgical mask.

Before Start

- ✓ It is highly recommended to read through the whole manual prior start for first time user.
- ✓ Make sure no precipitation observes in **TLB1 Buffer** and **TLB2 Buffer**. Dissolve the precipitation, if any, by incubating the bottle at 50 – 70°C before use.
- ✓ Before the nucleic acid extraction begins, set the desired temperature of water bath/ thermal block according to protocol.
- ✓ Add the indicated Ethanol (96 – 100%) and Proteinase Buffer to the respective component as follows:

P/No	Wash Buffer T2	Proteinase K
	Ethanol to be added	Proteinase Buffer to be added
KIT-9020-10	8 mL	260 µL
KIT-9020-50	48 mL	1.35 mL
KIT-9020-250	200 mL	3.35 mL

Sample Homogenization

There are 2 major methods for sample homogenization before the nucleic acid extraction begins. Select the method according to accessibility of your laboratory and please note that not all protocols require sample homogenization.

Method 1: Liquid Nitrogen (LN_2)

Pre-cool **mortar and pestle** using LN_2. Grind the frozen sample into fine powder with LN_2. Keep the sample frozen throughout the grinding process.

This is a preferred method for all nucleic acid extraction due to its versatility among different type of samples and works very well for small number of samples.

Method 2: Mechanical Homogenization

Option 1: **IKA Ultra-Turrax®**
Add the weighted tissue into 1.5 mL tube containing 50 – 75 µL phosphate buffered saline (PBS). Use the homogenizer to homogenize the sample into smaller pieces or fine powder.

Option 2: **Glass tube-homogenizer** (Cat# P7859-1EA, Sigma)
Add the weighted tissue into 1.5 mL tube containing 50 – 75 µL phosphate buffered saline (PBS). Sample is progressively ground smaller in the **rounded section using the PTFE pestle**. Homogenate is produced as the sample is forced through the cylindrical section. This is recommended for use with **soft tissues.**

Option 3: **Steel Beads, 3mm** (Cat# 740814.50, Macherey-Nagel)
Add the weighted tissue into 2 mL bead tube containing 5 pcs of 3 mm steel beads. Vortex at maximum speed for 5 to 20 minutes. The vortex speed and duration of vortex may vary according to sample type. Removal of the beads using strong magnet is optional.

A) Protocol – Human/ Animal Tissue

Reagent Supplied by User

✓ [Optional] 10 mg/mL RNase A solution

Sample	1. Transfer up to **25 mg human/ animal tissue** (small pieces) into a 1.5 mL tube. *Optional: Difficult samples are recommended to be homogenized using either LN_2 or mechanical homogenizer. Refer Page 5 for homogenization methods.*
Lysis	2. Add **180 µL TLB1 Buffer** and **25 µL Proteinase K Solution**. Vortex to mix thoroughly. ***Note***: *Make sure the sample is completely submerged in the solution.* 3. Incubate the sample at 56 °C for 1 to 3 hours/ overnight until lysis is completed. Vortex occasionally or use a shaking incubator. ***Note***: *1 hour incubation is recommended for first time user. Additional hours may be required to complete the lysis.* 4. **[Optional]** RNase Treatment: Add 20 µL RNase A Solution (not provided) and incubate for 5 min at room temperature. 5. Add **200 µL TLB2 Buffer** and vortex vigorously. 6. Incubate at 70 °C for 10 minutes. ***Note***: *If insoluble particles are observed, centrifuge for 5 minutes at 11,000 x g and transfer the supernatant to a new 1.5 mL tube.*

Binding	7. Add **210 µL of ethanol** (not provided) to the sample. Mix by vortex vigorously. ***Note:*** *Possibility of stringy precipitate may be observed.* 8. Place one **PrimeWay Genomic Column** into a Collection Tube. Transfer the lysate into the PrimeWay Genomic Column and centrifuge at 11,000 × *g* for 1 minute. ***Note:*** *Make sure to transfer all lysate into the column including precipitate in step 7 if any.* 9. Discard the Collection Tube and place the **PrimeWay Genomic Column** into a new Collection Tube. ***Note:*** *Repeat centrifugation if lysate did not completely flow-through the column.*
Washing	10. Add **500 µL Wash Buffer T1** into the column. Centrifuge at 11,000 × *g* for 1 minute. Discard the flow-through and place the column back into the Collection Tube. 11. Add **600 µL Wash Buffer T2** into the column. Centrifuge at 11,000 × *g* for 1 minute. Discard the flow-through and place the column back into the Collection Tube.
Drying	12. Centrifuge again at 11,000 × *g* for 1 minute to remove ethanol residue.
Elution	13. Place the **PrimeWay Genomic Column** into a new 1.5 mL centrifuge tube. 14. Add **100 µL Elution Buffer** to the center of the column membrane. Let the column stand at room temperature for 1 minute. 15. Centrifuge at 11,000 x *g* for 1 minute to elute the DNA.

B) Protocol – Cultured Cells

Sample	1. Resuspend **up to 10^7 cells** into final volume of **200 µL TLB1 Buffer**.
Lysis	2. Add **25 µL Proteinase K Solution**. 3. Add **200 µL TLB2 Buffer** and vortex to mix. 4. Incubate at 70 °C for 10 – 15 minutes.
Binding	5. Add **210 µL ethanol** (not provided) to the sample. Mix by vortex vigorously. ***Note:*** *Possibility of stringy precipitate may be observed.* 6. Place one **PrimeWay Genomic Column** into a Collection Tube. Transfer the lysate into the PrimeWay Genomic Column and centrifuge at 11,000 × *g* for 1 minute. ***Note:*** *Make sure to transfer all lysate into the column including precipitate in step 5 if any.* 7. Discard the Collection Tube and place the **PrimeWay Genomic Column** into a new Collection Tube. ***Note:*** *Repeat centrifugation if lysate did not completely flow-through the column.*
Washing	8. Add **500 µL Wash Buffer T1** into the column. Centrifuge at 11,000 × *g* for 1 minute. Discard the flow-through and place the column back into the Collection Tube. 9. Add **600 µL Wash Buffer T2** into the column. Centrifuge at 11,000 *x g* for 1 minute. Discard the flow-through and place the column back into the Collection Tube.

Drying	10. Centrifuge again at 11,000 *x g* for 1 minute to remove ethanol residue.
Elution	11. Place the PrimeWay Genomic Column into a new 1.5 mL centrifuge tube. 12. Add **100 µL Elution Buffer** to the center of the column membrane. Let the column stand at room temperature for 1 minute. 13. Centrifuge at 11,000 x *g* for 1 minute to elute the DNA.

C) Protocol – Mouse / Rat Tails

Sample	1.a) Mouse tails: Cut **2 pieces of 0.6 cm of mouse tail** and place it in a new 1.5 mL centrifuge tube. **OR** 1.b) Rat tail: Cut **1 piece 0.6 cm of rat tail** and place in a new 1.5 mL centrifuge tube.
Lysis	2. Add **180 µL TLB1 Buffer** and **25 µL Proteinase K Solution**. Vortex to mix thoroughly. 3. Incubate the sample at 56 °C for overnight until lysis is completed. Vortex occasionally or use a shaking incubator. 4. Centrifuge at 11,000 x *g* for 5 minutes. This is for removal of bones residual or hair. Transfer 200 µL supernatant to a new 1.5 mL tube. 5. Add **200 µL TLB2 Buffer** and vortex vigorously.
Binding	6. Add **210 µL ethanol** (not provided) to the sample. Mix by vortex vigorously. ***Note:*** *Possibility of stringy precipitate may be observed.* 7. Place one **PrimeWay Genomic Column** into a Collection Tube. Transfer the lysate into the PrimeWay Genomic Column and centrifuge at 11,000 × *g* for 1 minute. ***Note:*** *Make sure to transfer all lysate into the column including precipitate in step 6 if any.* 8. Discard the Collection Tube and place the **PrimeWay Genomic Column** into a new Collection Tube. ***Note:*** *Repeat centrifugation if lysate did not completely flow-through the column.*

Washing	9. Add **500 µL Wash Buffer T1** into the column. Centrifuge at 11,000 × *g* for 1 minute. Discard the flow-through and place the column back into the Collection Tube. 10. Add **600 µL Wash Buffer T2** into the column. Centrifuge at 11,000 × *g* for 1 minute. Discard the flow-through and place the column back into the Collection Tube.
Drying	11. Centrifuge again at 11,000 × *g* for 1 minute to remove ethanol residue.
Elution	12. Place the **PrimeWay Genomic Column** into a new 1.5 mL centrifuge tube. 13. Add **100 µL Elution Buffer** to the center of the membrane. Let the column stand at room temperature for 1 minute. 14. Centrifuge at 11,000 x *g* for 1 minute to elute the DNA.

D) Protocol – Bacteria

Reagents Supplied by User

- ✓ Gram-positive bacteria:
  - ➢ Bacteria Pre-Lysis Buffer [20 mM Tris-HCl; 2 mM EDTA; 1% Triton X-100, pH8]
  - ➢ Lysozyme
- ✓ [Optional] 10 mg/mL RNase A solution

Sample	1. Centrifuge up to **1 mL of bacteria culture** or **20 mg cell pellet** for 5 minutes at 8,000 × g. Remove the supernatant. Then, proceed to **2A for Gram-negative bacteria** **or** **2B for Gram-positive bacteria** accordingly.
Lysis (Gram-negative)	**2A) Gram-negative Bacteria** i) Resuspend the bacteria pellet in **180 µL TLB1 Buffer**. Add **25 µL Proteinase K solution** and vortex vigorously. ii) Incubate the sample at 56 °C for 1 to 3 hours/ overnight until lysis is complete. Vortex occasionally or use a shaking incubator. ***Note***: *1 hour incubation is recommended for first time user. Additional hours may be required to complete the lysis.* iii) **[Optional]** RNase Treatment: Add 40 µL RNase A Solution (not provided) and incubate for 5 minutes at room temperature. iv) Add **200 µL TLB2 Buffer** and vortex vigorously. Incubate at 70 °C for 10 minutes. ***Note***: *If insoluble particles are observed, centrifuge for 5 minutes 11,000 x g and transfer the supernatant to a new 1.5 mL tube.*

OR

Lysis (Gram-positive)

2B) Gram-positive Bacteria

i) Resuspend the bacteria pellet in **180 µL Bacteria Pre-Lysis Buffer** (not provided).

ii) Add **Lysozyme** with final concentration of 20 mg/mL (not provided) and incubate at 37 °C for 30 – 60 minutes.
Note: *Lysozyme can be pre-dissolved with Bacteria Pre-Lysis Buffer before step 2B(i). E.g., dissolve 20 mg of lyophilized lysozyme in 1 mL Bacteria Pre-Lysis Buffer*

iii) Add **25 µL Proteinase K Solution** and vortex to mix thoroughly.

iv) Incubate the sample at 56 °C for 1 to 3 hours/ overnight until lysis is complete. Vortex occasionally or use a shaking incubator.
Note: *1 hour incubation is recommended for first time user. Additional hours may be required to complete the lysis.*

v) **[Optional]** RNase Treatment: Add 40 µL RNase A Solution (not provided) and incubate for 5 minutes at room temperature.

vi) Add **200 µL TLB2 Buffer** and vortex vigorously. Incubate at 70 °C for 10 minutes.
Note: *If insoluble particles are observed, centrifuge for 5 minutes at 11,000 x g and transfer the supernatant to a new 1.5 mL tube.*

Binding

3. Add **210 µL ethanol** (not provided) to the sample. Mix by vortex vigorously.
Note: *Possibility of stringy precipitate may be observed.*

4. Place one **PrimeWay Genomic Column** into a Collection Tube. Transfer the lysate into the PrimeWay Genomic Column and centrifuge at 11,000 × *g* for 1 minute.
Note: *Make sure to transfer all lysate into the column including precipitate in step 3 if any.*

Binding	5. Discard the Collection Tube and place the **PrimeWay Genomic Column** into a new Collection Tube. ***Note**: Repeat centrifugation if lysate did not completely flow-through the column.*
Washing	6. Add **500 µL Wash Buffer T1** into the column. Centrifuge at 11,000 × *g* for 1 minute. Discard the flow-through and place the column back into the Collection Tube. 7. Add **600 µL Wash Buffer T2** into the column. Centrifuge at 11,000 × *g* for 1 minute. Discard the flow-through and place the column back into the Collection Tube.
Drying	8. Centrifuge again at 11,000 × *g* for 1 minute to remove ethanol residue.
Elution	9. Place the **PrimeWay Genomic Column** into a new 1.5 mL centrifuge tube. 10. Add **100 µL Elution Buffer** to the center of the column membrane. Let the column stand at room temperature for 1 minute. 11. Centrifuge at 11,000 x *g* for 1 minute to elute the DNA.

E) Protocol – Yeast / Fungi

Reagents Supplied by User

- ✓ Prepare 10 mM EDTA, pH 8.0 working solution
- ✓ Prepare Sorbitol Buffer [1.2 M Sorbitol; 10 mM $CaCl_2$; 0.1 M Tris-HCl pH 7.5] and store at 4°C, add in 35 mM β-mercaptoethanol before use
- ✓ Prepare 200 U Zymolyase working solution
- ✓ [Optional] 10 mg/mL RNase A solution

Sample	1. Centrifuge **3 mL of YPD yeast culture (5 – 6 x 10^7)** or **30 mg cell pellet** at 5,000 x *g* for 10 minutes. Remove the supernatant. 2. Wash the cells pellet with **1 mL 10mM EDTA, pH 8** (not provided). 3. Centrifuge at 5,000 x *g* for 10 minutes and remove the supernatant.
Lysis	4. Resuspend the pellet in **600 µL Sorbitol Buffer** (not provided, add in 35 mM β-mercaptoethanol before use). 5. Add **200 U Zymolyase** (not provided). Incubate at 30 °C for 30 minutes to degrade yeast cell wall to form spheroplasts. 6. Centrifuge at 2,000 × *g* for 10 minutes to remove supernatant. 7. Resuspend the spheroplasts with **180 µL TLB1 Buffer.** Add **25 µL Proteinase K solution** and vortex vigorously to mix. 8. Incubate the sample at 56 °C for 1 to 3 hours/ overnight until lysis is completed. Vortex occasionally or use a shaking incubator. ***Note***: *1 hour incubation is recommended for first time user. Additional hours may be required to complete the lysis.* 9. **[Optional]** RNase Treatment: Add 40 µL RNase A (not provided) and incubate for 5 minutes at room temperature.

Lysis	10. Add **200 µL TLB2 Buffer** and vortex vigorously. Incubate at 70 °C for 10 minutes. ***Note**: If insoluble particles are observed, centrifuge for 5 minutes at 11,000 x g and transfer the supernatant to a new 1.5 mL tube.*
Binding	11. Add **210 µL ethanol** (not provided) to the sample. Mix by vortex vigorously. ***Note**: Possibility of stringy precipitate may be observed.* 12. Place one **PrimeWay Genomic Column** into a Collection Tube. Transfer the lysate into the PrimeWay Genomic Column and centrifuge at 11,000 × *g* for 1 minute. ***Note**: Make sure to transfer all lysate into the column including precipitate in step 11 if any.* 13. Discard the Collection Tube and place the **PrimeWay Genomic Column** into a new Collection Tube. ***Note**: Repeat centrifugation if lysate did not completely flow-through the column.*
Washing	14. Add **500 µL Wash Buffer T1** into the column. Centrifuge at 11,000 × *g* for 1 minute. Discard the flow-through and place the column back into the Collection Tube. 15. Add **600 µL Wash Buffer T2** into the column. Centrifuge at 11,000 × *g* for 1 minute. Discard the flow-through and place the column back into the Collection Tube.
Drying	16. Centrifuge again at 11,000 × *g* for 1 minute to remove ethanol residue.

Elution	
	17. Place the **PrimeWay Genomic Column** into a new 1.5 mL centrifuge tube. 18. Add **100 µL Elution Buffer** to the center of the column membrane. Let the column stand at room temperature for 1 minute. 19. Centrifuge at 11,000 x *g* for 1 minute to elute the DNA.

F) Protocol – Blood on FTA Card

Sample	1. Cut **1 or 2 dried blood spots** and place it in a 1.5 mL centrifuge tube. ***Note:*** *Each blood spot area shall be between 15 – 30 mm².*
Lysis	2. Add **180 µL TLB1 Buffer** and mix by vortex. Incubate the sample at 94 °C for 10 minutes. Cool the sample to room temperature. 3. Add **25 µL Proteinase K Solution** and vortex briefly to mix. 4. Incubate the sample at 56 °C for 1 hour. Vortex occasionally or use a shaking incubator. ***Note:*** *Ensure the samples are submerged and covered with buffer.* 5. Add **200 µL TLB2 Buffer** and vortex vigorously. 6. Incubate at 56 °C for 10 minutes.
Binding	7. Add **210 µL ethanol** (not provided) to the sample. Mix by vortex vigorously. ***Note:*** *Possibility of stringy precipitate may be observed.* 8. Place one **PrimeWay Genomic Column** into a Collection Tube. Transfer the lysate into the PrimeWay Genomic Column and centrifuge at 11,000 × *g* for 1 minute. ***Note:*** *Make sure to transfer all lysate into the column including precipitate in step 7 if any.* 9. Discard the Collection Tube and place the **PrimeWay Genomic Column** into a new Collection Tube. ***Note:*** *Repeat centrifugation if lysate did not completely flow-through the column.*

Washing	10. Add **500 µL Wash Buffer T1** into the column. Centrifuge at 11,000 × *g* for 1 minute. Discard the flow-through and place the column back into the Collection Tube. 11. Add **600 µL Wash Buffer T2** into the column. Centrifuge at 11,000 × *g* for 1 minute. Discard the flow-through and place the column back into the Collection Tube.
Drying	12. Centrifuge again at 11,000 × *g* for 1 minute to remove ethanol residue.
Elution	13. Place the **PrimeWay Genomic Column** into a new 1.5 mL centrifuge tube. 14. Add **100 µL Elution Buffer** to the center of the column membrane. Let the column stand at room temperature for 1 minute. 15. Centrifuge at 11,000 x *g* for 1 minute to elute the DNA.

G) Protocol – Blood

Sample	1. Equilibrate the blood or body fluid sample from EDTA tube at room temperature. 2. Transfer **up to 200 µL of sample** into a new 1.5 mL microcentrifuge tube. ***Note**: If sample ≤ 200 µL, top up the volume with 1xPBS (not provided) to 200 µL.*
Lysis	3. Add **25 µL Proteinase K** to the samples. 4. Add **200 µL TLB2 Buffer** and vortex vigorously for 10 – 20 seconds to mix. 5. Incubate at room temperature for 5 minutes. Mix by vortex. 6. Incubate the samples at 70 °C for 10 – 15 minutes. ***Note**: Brownish solution should be observed during incubation. If processing older or clotted blood samples, increase incubation time up to 30 minutes & vortex once or twice vigorously during incubation.*
Binding	7. Add **210 µL ethanol** (not provided) to the sample. Mix by vortex. 8. Place one **PrimeWay Genomic Column** into a Collection Tube. Transfer the lysate into the PrimeWay Genomic Column and centrifuge at 11,000 × *g* for 1 minute. ***Note**: Repeat centrifugation at higher speed (≤ 15,000 x g) if lysate did not completely flow-through the column.* 9. Discard the Collection Tube and place the **PrimeWay Genomic Column** into a new Collection Tube.

Washing	10. Add **500 µL Wash Buffer T1** into the column. Centrifuge at 11,000 × *g* for 1 minute. Discard the flow-through and place the column back into the Collection Tube. 11. Add **600 µL Wash Buffer T2** into the column. Centrifuge at 11,000 × *g* for 1 minute. Discard the flow-through and place the column back into the Collection Tube.
Drying	12. Centrifuge again at 11,000 × *g* for 1 minute to remove ethanol residue.
Elution	13. Place the **PrimeWay Genomic Column** into a new 1.5 mL centrifuge tube. 14. Add **100 µL Elution Buffer** to the center of the column membrane. Let the column stand at room temperature for 1 minute. 15. Centrifuge at 11,000 x *g* for 1 minute to elute the DNA.

H) NGS Grade DNA Extraction Protocol – Buffy coat

Reagents Supplied by User

- ✓ 10 mg/mL RNase A solution
- ✓ Chloroform

Sample	1. Thaw the frozen buffy coat in water bath at 37 °C. Proceed to extraction immediately. 2. Transfer all **buffy coat (~200 – 250 µL)** into a new 1.5 mL microcentrifuge tube.
Lysis	3. Add **25 µL Proteinase K** and **40 µL RNase A Solution** to the samples. 4. Add **200 µL TLB2 Buffer** and vortex briefly to mix. 5. Allow the sample lysis at room temperature for 5 minutes. 6. Inactivate the enzymes by incubating the mixture at 70°C for 15 minutes. 7. Add equal volume of **chloroform** (~500 µL) and shake vigorously by hand to mix. 8. Centrifuge at maximum speed (16,000 x *g*) for 10 minutes. 9. Transfer the aqueous phase (~210 µL) into a new 1.5 mL microcentrifuge tube. ***Note**: Do not disturb the interphase.*

Binding	10. Add **equal volume of ethanol** (not provided) to the sample. Shake vigorously by hand to mix. 11. Place one **PrimeWay Genomic Column** into a Collection Tube. Transfer the lysate into the PrimeWay Genomic Column and centrifuge at 11,000 × *g* for 1 minute. ***Note**: Repeat centrifugation at higher speed (≤ 15,000 x g) if lysate did not completely flow-through the column.* 12. Discard the Collection Tube and place the **PrimeWay Genomic Column** into a new Collection Tube.
Washing	13. Add **500 µL Wash Buffer T1** into the column. Centrifuge at 11,000 × *g* for 1 minute. Discard the flow-through and place the column back into the Collection Tube. 14. Add **600 µL Wash Buffer T2** into the column. Centrifuge at 11,000 × *g* for 1 minute. Discard the flow-through and place the column back into the Collection Tube.
Drying	15. Centrifuge again at 11,000 × *g* for 1 minute to remove ethanol residue.
Elution	16. Place the **PrimeWay Genomic Column** into a new 1.5 mL centrifuge tube. 17. Add **100 µL Elution Buffer** to the center of the column membrane. Let the column stand at room temperature for 1 minute. 18. Centrifuge at 11,000 x *g* for 1 minute to elute the DNA.

I) Protocol – Insects

Reagent Supplied by User

✓ [Optional] 10 mg/mL RNase A solution

Sample	1. Homogenize ~ **50 mg insects** using either LN_2 or mechanical homogenizer. Refer Page 5 for homogenization methods. ***Note***: *It is recommended to store whole body of insects in 5x volume of ethanol before homogenization.* 2. Transfer the homogenized tissues into 1.5 mL centrifuge tube.
Lysis	3. Add **180 µL TLB1 Buffer** and **25 µL Proteinase K solution**. Vortex to mix thoroughly. ***Note***: *Make sure the sample is completely submerged in the solution.* 4. Incubate the sample at 56 °C for 1 to 3 hours/ overnight until lysis is complete. Vortex occasionally or use a shaking incubator. ***Note***: *1 hour incubation is recommended for first time user. Additional hours may be required to complete the lysis.* 5. [Optional] RNase Treatment: Add 20 µL RNase A (not provided) and incubate for 5 min at room temperature. 6. Add **200 µL TLB2 Buffer** and vortex vigorously. 7. Incubate at 70 °C for 10 minutes. ***Note***: *If insoluble particles are observed, centrifuge for 5 minutes at 11,000 x g and transfer the supernatant to a new 1.5 mL tube.*

Binding	8. Add **210 µL ethanol** (not provided) to the sample. Mix by vortex vigorously. ***Note:*** *Possibility of stringy precipitate may be observed.* 9. Place one **PrimeWay Genomic Column** into a Collection Tube. Transfer the lysate into the PrimeWay Genomic Column and centrifuge at 11,000 × *g* for 1 minute. ***Note:*** *Make sure to transfer all lysate into the column including precipitate in step 8 if any.* 10. Discard the Collection Tube and place the **PrimeWay Genomic Column** into a new Collection Tube. ***Note:*** *Repeat centrifugation if lysate did not completely flow-through the column.*
Washing	11. Add **500 µL Wash Buffer T1** into the column. Centrifuge at 11,000 × *g* for 1 minute. Discard the flow-through and place the column back into the Collection Tube. 12. Add **600 µL Wash Buffer T2** into the column. Centrifuge at 11,000 × *g* for 1 minute. Discard the flow-through and place the column back into the Collection Tube.
Drying	13. Centrifuge again at 11,000 × *g* for 1 minute to remove ethanol residue.
Elution	14. Place the **PrimeWay Genomic Column** into a new 1.5 mL centrifuge tube. 15. Add **100 µL Elution Buffer** to the center of the column membrane. Let the column stand at room temperature for 1 minute. 16. Centrifuge at 11,000 x *g* for 1 minute to elute the DNA.

J) Protocol – Buccal Swabs

Reagent Supplied by User

✓ 1x Phosphate Buffered Saline (PBS), Biotechnology Grade

Sample	1. Firmly scrape the inner cheek several times using buccal swab tool. ***Note:*** *Do not consume any food or drink 30 minutes prior of sample collection.* 2. Air dry the swab.
Lysis	3. Transfer the dry swab into a 2 mL centrifuge tube. Add **400 – 600 µL of 1x PBS Buffer** (not provided) and **25 µL Proteinase K solution** into the tube containing the swab. 4. Mix by vortex for 5 seconds. Repeat vortex, for a total of 2 times. Incubate at 56 °C for 10 minutes. 5. Discard the swab, transfer all the lysate solution to a new 1.5 mL centrifuge tube. 6. Add ***one volume TLB2 Buffer** and vortex vigorously to mix. **For example: 400 µL PBS used in Step 3, add 400 µL TLB2 Buffer.* 7. Incubate the lysate at 70 °C for 10 minutes.
Binding	8. Add ***one volume of ethanol** (not provided) to the lysate and vortex vigorously to mix. **For example: 400 µL PBS used in Step 3, add 400 µL ethanol (96 – 100%).* 9. Place one **PrimeWay Genomic Column** into a Collection Tube.

Binding	10. Transfer the **600 µL lysate** into the **PrimeWay Genomic Column** and centrifuge at 11,000 × *g* for 1 minute. ***Note**: Repeat centrifugation if lysate did not completely flow-through the column.* 11. Discard the flow-through and place the **PrimeWay Genomic Column** back into Collection Tube. 12. Repeat Step 10 and 11 until all lysates pass through the column. 13. Discard the Collection Tube and place the **PrimeWay Genomic Column** into a new Collection Tube.
Washing	14. Add **500 µL Wash Buffer T1** into the column. Centrifuge at 11,000 × *g* for 1 minute. Discard the flow-through and place the column back into the Collection Tube. 15. Add **600 µL Wash Buffer T2** into the column. Centrifuge at 11,000 × *g* for 1 minute. Discard the flow-through and place the column back into the Collection Tube.
Drying	16. Centrifuge again at 11,000 × *g* for 1 minute to remove ethanol residue.
Elution	17. Place the **PrimeWay Genomic Column** into a new 1.5 mL centrifuge tube. 18. Add **100 µL Elution Buffer** to the center of the column membrane. Let the column stand at room temperature for 1 minute. 19. Centrifuge at 11,000 x *g* for 1 minute to elute the DNA.

Troubleshooting Guidelines

Problems	Possible Reason	Recommended Action
Low DNA yield	Poor homogenization of sample	Incomplete lysis of sample with TLB1 Buffer or Proteinase K. Vortex the mixture vigorously immediately after adding TLB1 Buffer.
	Reagents not applied appropriately	Make sure preparation of Wash Buffer T2 and Proteinase K solution is according to the protocol in "Before Start", refer page 4.
		Add ethanol accordingly to the lysates before loading them onto columns.
	DNA Elution	Preheat the Elution Buffer to 70 °C before elution step and apply directly to the center of membrane.
		Recommend increased of Elution buffer to 200 µL and incubate with closed column at 70 °C for 5 minutes before centrifuge.
PrimeWay Genomic Column is clogged	Too much sample materials	Recommended to follow sample material suggested. Centrifuge to pellet down the debris/ insoluble material. Transfer the supernatant to a new 1.5 mL microcentrifuge tube prior addition of TLB2 and ethanol.
	Incomplete lysis	Sample not thoroughly homogenized with TLB1 Buffer or Proteinase K. Vortex the mixture vigorously immediately after adding TLB1 Buffer.
		Proteinase K activity decreased. Make sure proper storage.
DNA quality is poor	Salt or ethanol carry over	Ensure Drying Step is performed accordingly to ensure column membrane is dry. Repeat centrifugation if necessary.
	Reagents not applied appropriately	Make sure preparation of Wash Buffer T2 and Proteinase K solution is according to the protocol in "Before Start", refer page 4. Add ethanol accordingly to the lysates before loading them onto columns
	Presence of RNA	Perform RNase treatment. Add 20 µL RNase A [10 mg/mL; supplied by users] and incubate for 5 minutes at 37 °C before addition of TLB2 Buffer.

Please contact us at https://base-asia.com/contact/ for more information.

Product Ordering Information

Sample Type	Part Number	Product Description	Remarks
Human/ Animal Tissue, Bacteria, Yeast/ Fungi, Buffy Coat, Insect	1st BASE K.RGT-9104-1ml	RNase A Solution, 10mg/mL, 1mL	Each vial sufficient for: ✓ 25 preps (bacteria, yeast/ fungi & buffy coat) ✓ 50 preps (human/ animal tissue & insect)
Bacteria (Gram +ve)	1st BASE K.RGT-9108-110mg	Lysozyme, 110 mg/vial, 1 vial	Each vial sufficient for 30 preps
	1st BASE K.BUF-9105-50ml	Bacterial Pre-Lysis Buffer, 50mL	Sufficient for 250 preps
Yeast/ Fungi	1st BASE BUF-1053-100ml-pH8.0	0.5M EDTA solution, pH 8.0, Biotechnology Grade, 100mL	Dilute from 500mM stock solution to 10mM working solution before use.
	1st BASE K.RGT-9107-20000U	Zymolyase(R)-20T, 20000 U/g, 1g	Sufficient for 100 preps
	1st BASE BUF-1416-1L-pH7.5	1M Tris-HCl, pH7.5, Biotechnology Grade, 1L	Sorbitol Buffer Recipe *Short product stability: 6 months at 4°C due to the presence of sorbitol.
	Nacalai Tesque 32020-05	Sorbitol = D-Glucitol, EP Grade, 500G	
	Nacalai Tesque 08894-25	Calcium Chloride, SP Grade for Molecular Biology, 500G	
	Nacalai Tesque 21438-82	2-Mercaptoethanol, SP Grade for Molecular Biology, 25G	Sorbitol Buffer Recipe *To freshly add into Sorbitol buffer before use
Buffy Coat	Nacalai Tesque 16487-02	Chloroform, SP Grade for Nucleic acid Extraction, 25mL	
Buccal Swabs	1st BASE BUF-2041-1x1L	1X Phosphate Buffered Saline (PBS), Biotechnology Grade, 1L	

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