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Ver. 2.0

PrimeWay Genomic II DNA Extraction Kit (KIT-9022)

Sample Types

- Animal Tissue
- Cultured Cells
- Mouse/ Rat Tail
- Bacteria
- Yeast
- Fungi (cut agar)
- Blood
- White Blood Cells
- Insects
- Buccal Swab
- Saliva
- Alcohol-fixed Tissue
- Sperm
- Feathers/ Nails/ Hair

Molecular Biology Kit

PrimeWay Genomic II DNA Extraction Kit

Product No: KIT-9022

PrimeWay Genomic II DNA Extraction Kit is a rapid and reliable kit that isolates high quality genomic DNA from 16 sample types, including animal tissue, cultured cells, mouse/rat tail, bacteria, yeast, fungi from cut agar, whole blood, buffy coat, nucleated blood, dried blood spot, white blood cells, insects, buccal swab, saliva, alcohol-fixed tissue, sperm and feathers/ nails/ hair.

This kit uses both chemical (SDS) and enzymatic lysis (Proteinase K) to effectively isolate and purify high quality DNA. The extracted DNA is suitable for downstream applications such as genotyping, PCR, restriction analysis, Southern blotting, etc. The extracted DNA from cultured cells, bacteria, yeast, whole blood, buffy coat, nucleated blood and white blood cells are suitable for Next-Generation Sequencing.

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

Kit Contents

No	Product	KIT-9022-10 10 preps*	KIT-9022-50 50 preps*	KIT-9022-250 250 preps*
1	GL1 Buffer	2.5 mL	12 mL	60 mL
2	GL2 Buffer	2.5 mL	12 mL	60 mL
3	Wash Buffer G1	4.5 mL	22 mL	110 mL
4	Wash Buffer G2	1.5 mL	7 mL	35 mL
5	Elution Buffer	1.5 mL	6 mL	30 mL
6	Proteinase K	10 mg	25 mg	110 mg
7	Proteinase K Buffer	550 µL	1.3 mL	5.75 mL
8	RNase A Solution	50 µL	250 µL	1.1 mL
9	PrimeWay Genomic II Column	10 pcs	50 pcs	5 x 50 pcs
10	Collection Tube	2 x 10 pcs	2 x 50 pcs	10 x 50 pcs

* Number of preps are different for certain sample types. Refer to **Table B** for more information.

Storage

This kit will be delivered at room temperature (21 – 25 °C). Store the kit at room temperature (21 – 25 °C). Once RNase A Solution is opened and Proteinase K is reconstituted, store them at −20 °C.

Product Specification

	KIT-9022
Binding capacity	50 µg
Yield	Up to 40 µg
Sample Size	Refer **Table A**
Elution	50 – 100 µL
Duration	~25 minutes/prep (exclude lysis incubation)

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

Protocol	Sample Type	Sample Size	Page
A	Animal tissue	Up to 25 mg	6 – 7
B	Cultured cells	Up to 1 x 10^7 cells	8 – 9
C	Mouse/ Rat tail	Up to 25 mg tail or ≤ 2 pcs, 0.5 cm tail	10 – 11
D	Bacteria	Up to 20 mg	12 – 14
E	Yeast	Up to 30 mg	15 – 17
F	Fungi from cut agar	0.5 cm x 0.5 cm cut agar	18 – 20
G	Whole blood/ Buffy coat	200 µL	21 – 22
H	Nucleated blood#	10 µL	23 – 24
I	Dried blood spot	≤ 2 Spots (15 – 30 mm^2 each)	25 –26
J	White blood cells (Leukocytes)	Up to 1 mL	27 – 29
K	Insects	Up to 50 mg	30 – 31
L	Buccal swab	1 swab	32 – 33
M	Saliva	1 mL	34 – 35
N	Alcohol-fixed tissue	≤ 25 mg	36 – 37

O	Sperm	100 µL	38 – 39
P	Feathers/ Nails/ Hair	Feather: 2 – 5 quills Nail: 10 – 25 mg Hair: 1 – 10 strands hair with follicle	40 – 41

[#] Examples of organisms with nucleated blood: birds and fish

Table B: *Certain sample type is only sufficient for the number of preps as stated below.*

Protocol	Sample Type	KIT-9022-10	KIT-9022-50	KIT-9022-250
I	Dried blood spot	6 preps	30 preps	150 preps
L	Buccal swab	6 preps	30 preps	150 preps
P	Feathers/ Nails/ Hair	8 preps	40 preps	200 preps

Materials Supplied by User

- ✓ Undenatured absolute ethanol (≥ 99.5%)
- ✓ Nuclease-free water
- ✓ Vortex mixer
- ✓ Centrifuge, at speed of 2,000 – 16,000 x *g*
- ✓ Water bath or dry bath
- ✓ Pipettes & pipette tips
- ✓ 1.5 mL microcentrifuge tubes
- ✓ 2 mL microcentrifuge tubes (white blood cells & buccal swab only)
- ✓ 15 mL & 50 mL centrifuge tube (saliva only)
- ✓ EDTA tube (whole blood/ buffy coat & white blood cells only)
- ✓ Liquid nitrogen (animal tissue & insects only)
- ✓ Mortar and pestle (animal tissue & insects only)
- ✓ Thermomixer (yeast, fungi from cut agar & dried blood spot only)
- ✓ Refer **Table C** for the additional reagents required for different sample types

***Table C**: Additional reagents supplied by users.*

Protocol	Sample Type	Reagents
B	Cultured cells	✓ 1X Phosphate Buffered Saline (PBS)
D	Bacteria (Gram-positive bacteria)	✓ Bacteria Pre-Lysis Buffer ✓ Lysozyme ✓ 50 mM EDTA, pH 8.0
E	Yeast	✓ 10 mM EDTA, pH 8.0 ✓ Sorbitol Buffer *[1.2 M Sorbitol; 10 mM $CaCl_2$; 0.1 M Tris-HCl pH 7.5]* ✓ β-mercaptoethanol (β-Me) ✓ Zymolyase
F	Fungi from cut agar	✓ Sorbitol Buffer *[1.2 M Sorbitol; 10 mM $CaCl_2$; 0.1 M Tris-HCl pH 7.5]* ✓ β-mercaptoethanol (β-Me) ✓ Zymolyase
G	Whole blood/ Buffy coat	✓ 1X Phosphate Buffered Saline (PBS)
H	Nucleated blood	✓ 1X Phosphate Buffered Saline (PBS)
J	White blood cells (Leukocytes)	✓ RBC Lysis Buffer ✓ 1X Phosphate Buffered Saline (PBS)
L	Buccal swab	✓ 1X Phosphate Buffered Saline (PBS)
M	Saliva	✓ 1X Phosphate Buffered Saline (PBS)
N	Alcohol-fixed Tissue	✓ 1X Phosphate Buffered Saline (PBS)
O	Sperm	✓ Sperm Lysis Buffer ✓ 1 M Dithiothreitol (DTT) Solution
P	Feather/ Nail/ Hair	✓ 1 M Dithiothreitol (DTT) Solution

Precautions for User

- ✓ Some buffers in this kit contain 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 to starting especially for first-time user.
- ✓ Proteinase K Solution preparation: Add indicated volume of Proteinase K Buffer to dissolve Proteinase K.

P/No	Proteinase K Buffer to be added
KIT-9022-10	500 µL
KIT-9022-50	1.25 mL
KIT-9022-250	5.5 mL

After reconstitution, store at –20 °C.

- ✓ Add absolute ethanol (≥ 99.5%) to Wash Buffer G2 as following:

P/No	Ethanol to be added
KIT-9022-10	6 mL
KIT-9022-50	28 mL
KIT-9022-250	140 mL

Sample Homogenisation

Pre-cool **mortar and pestle** using liquid nitrogen (LN_2). Freeze tissue samples immediately in LN_2 and grind the sample into fine powder under LN_2. Keep the sample frozen throughout the grinding process. Transfer the ground sample into a 1.5 mL microcentrifuge tube. Snap freezes the sample in LN_2 and store the samples in –80 °C. The samples are stable up to 6 months.

A) Protocol – Animal Tissue

Preparation	I. Set water bath/ dry bath to 60 °C. II. Preheat the **Elution Buffer** at 60 °C.
Sample	1. Grind tissue sample to fine powder with liquid nitrogen using mortar and pestle. *Refer page 5 for details of Sample Disruption.* 2. Transfer up to **25 mg of tissue powder** to a new 1.5 mL microcentrifuge tube. ***Note:*** *For tissue samples with higher number of cells (e.g., liver or spleen), reduce the sample input to 10 mg.*
Lysis	3. Add **200 µL GL1 Buffer** and **20 µL Proteinase K Solution**. Vortex to mix. 4. Incubate the sample at 60 °C for 3 hours/ overnight. Invert the tube occasionally. 5. Centrifuge at 14,000 x *g* for 2 minutes to pellet insoluble debris. 6. Transfer the supernatant to a new 1.5 mL microcentrifuge tube. 7. Add **200 µL GL2 Buffer**. Vortex to mix. 8. Add **4 µL RNase A Solution**. Vortex to mix and incubate at room temperature for 5 minutes.

Binding	9. Add **200 µL absolute ethanol** (not provided). Vortex to mix immediately. 10. Place a **PrimeWay Genomic II Column** into a new Collection Tube. 11. Transfer **up to 750 µL lysate**, including the precipitate if any, to the **PrimeWay Genomic II Column**. Centrifuge at 14,000 x *g* for 1 minute. 12. Discard the Collection Tube and place the column into a new Collection Tube.
Washing	13. Add **400 µL Wash Buffer G1** to the column. Centrifuge at 14,000 x *g* for 30 seconds. Discard the flow-through and place the column back into the Collection Tube. 14. Add **600 µL Wash Buffer G2** to the column. Centrifuge at 14,000 x *g* for 30 seconds. Discard the flow-through and place the column back into the Collection Tube.
Drying	15. Centrifuge the column at 14,000 x *g* for 3 minutes to dry the membrane.
Elution	16. Transfer the **PrimeWay Genomic II Column** to a new 1.5 mL microcentrifuge tube. 17. Add **100 µL preheated Elution Buffer** to the centre of the column membrane. Incubate at room temperature for at least 3 minutes. 18. Centrifuge at 14,000 x *g* for 30 seconds to elute the DNA.

B) Protocol – Cultured Cells

Reagent Supplied by User

✓ 1X Phosphate Buffered Saline (PBS)

Preparation	I. Set water bath/ dry bath to 60 °C. II. Preheat the **Elution Buffer** at 60 °C.
Lysis	1. Resuspend up to **1** x **10^7 cells** with **200 µL PBS** (not provided). 2. Add **20 µL Proteinase K Solution**. Vortex to mix and incubate at 60 °C for 5 minutes. 3. Add **200 µL GL2 Buffer**. Vortex to mix and incubate at 60 °C for 10 minutes. Invert tube every 5 minutes to mix. 4. Add **4 µL RNase A Solution**. Vortex to mix and incubate at room temperature for 5 minutes.
Binding	5. Add **200 µL absolute ethanol** (not provided). Vortex to mix immediately. 6. Place a **PrimeWay Genomic II Column** into a new Collection Tube. 7. Transfer **up to 750 µL lysate**, including the precipitate if any, to the **PrimeWay Genomic II Column**. Centrifuge at 14,000 x *g* for 1 minute. 8. Discard the Collection Tube and place the column into a new Collection Tube.

Washing	9. Add **400 µL Wash Buffer G1** to the column. Centrifuge at 14,000 x *g* for 30 seconds. Discard the flow-through and place the column back into the Collection Tube. 10. Add **600 µL Wash Buffer G2** to the column. Centrifuge at 14,000 x *g* for 30 seconds. Discard the flow-through and place the column back into the Collection Tube.
Drying	11. Centrifuge the column at 14,000 x *g* for 3 minutes to dry the membrane.
Elution	12. Transfer the **PrimeWay Genomic II Column** to a new 1.5 mL microcentrifuge tube. 13. Add **100 µL preheated Elution Buffer** to the centre of the column membrane. Incubate at room temperature for at least 3 minutes. 14. Centrifuge at 14,000 x *g* for 30 seconds to elute the DNA.

C) Protocol – Mouse / Rat Tail

Preparation	I. Set water bath/ dry bath to 60 °C. II. Preheat the **Elution Buffer** at 60 °C.
Sample	1A) Mouse tail: Excise **2 pieces of 0.5 cm** or up to **25 mg of mouse tail** and transfer to a new 1.5 mL microcentrifuge tube. **OR** 1B) Rat tail: Excise **1 piece of 0.5 cm** or up to **25 mg of rat tail** and transfer to a new 1.5 mL microcentrifuge tube.
Lysis	2. Add **200 µL GL1 Buffer** and **20 µL Proteinase K Solution**. Vortex to mix. 3. Incubate the sample at 60 °C for 3 hours/ overnight. Invert the tube occasionally. 4. Centrifuge at 14,000 x *g* for 2 minutes to pellet insoluble debris. 5. Transfer the supernatant to a new 1.5 mL microcentrifuge tube. 6. Add **200 µL GL2 Buffer**. Vortex to mix.
Binding	7. Add **200 µL absolute ethanol** (not provided). Vortex to mix immediately. 8. Place a **PrimeWay Genomic II Column** into a new Collection Tube. 9. Transfer **up to 750 µL lysate**, including the precipitate if any, to the **PrimeWay Genomic II Column**. Centrifuge at 14,000 x *g* for 1 minute.

Binding	10. Discard the Collection Tube and place the column into a new Collection Tube.
Washing	11. Add **400 µL Wash Buffer G1** to the column. Centrifuge at 14,000 x *g* for 30 seconds. Discard the flow-through and place the column back into the Collection Tube. 12. Add **600 µL Wash Buffer G2** to the column. Centrifuge at 14,000 x *g* for 30 seconds. Discard the flow-through and place the column back into the Collection Tube.
Drying	13. Centrifuge the column at 14,000 x *g* for 3 minutes to dry the membrane.
Elution	14. Transfer the **PrimeWay Genomic II Column** to a new 1.5 mL microcentrifuge tube. 15. Add **100 µL preheated Elution Buffer** to the centre of the column membrane. Incubate at room temperature for at least 3 minutes. 16. Centrifuge at 14,000 x *g* for 30 seconds 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; pH 8.0]
- Lyophilised lysozyme
- 50 mM EDTA, pH 8.0

Preparation	I. Set water bath/ dry bath to 60 °C. II. Preheat the **Elution Buffer** at 60 °C. III. <u>Gram-positive bacteria</u> **Bacteria Pre-Lysis Buffer Mix** preparation: Dissolve **20 mg Lysozyme** (not provided) into **1 mL Bacteria Pre-Lysis Buffer** (not provided).
Sample	1. Harvest **20 mg cell pellet** or centrifuge up to **1 mL of bacteria culture** at 8,000 x *g* for 5 minutes. Discard supernatant.
Lysis (Gram-negative)	**2A) Gram-negative Bacteria** i) Resuspend the pellet with **200 µL GL1 Buffer.** ii) Add **20 µL Proteinase K Solution**. Vortex to mix. iii) Incubate at 60 °C for 60 minutes. Invert the tube occasionally. iv) Add **200 µL GL2 Buffer**. Vortex to mix. v) Add **4 µL of RNase A Solution**. Vortex to mix and incubate at room temperature for 5 minutes. **OR**

Lysis (Gram-positive)	**2B) Gram-positive Bacteria** i) Resuspend the pellet with **420 µL 50 mM EDTA** (not provided). ii) Add **180 µL freshly prepared Bacteria Pre-Lysis Buffer mix** (not provided, refer previous page for preparation method). Incubate at 37 °C for 30 – 60 minutes. iii) Centrifuge at 16,000 x *g* for 2 minutes. Discard supernatant. iv) Resuspend the pellet with **200 µL GL1 Buffer**. v) Add **20 µL Proteinase K Solution**. Vortex to mix and incubate at 60 °C for 60 minutes. Invert the tube occasionally. vi) Add **200 µL GL2 Buffer**. Vortex to mix. vii) Add **4 µL of RNase A Solution**. Vortex to mix and incubate at room temperature for 5 minutes.
Binding	3. Add **200 µL of absolute ethanol** (not provided). Vortex to mix immediately. 4. Place a **PrimeWay Genomic II Column** into a new Collection Tube. 5. Transfer **up to 750 µL lysate**, including the precipitate if any, to the **PrimeWay Genomic II Column.** Centrifuge at 14,000 x *g* for 1 minute. 6. Discard the Collection Tube and place the column into a new Collection Tube.

Washing	7. Add **400 µL Wash Buffer G1** to the column. Centrifuge at 14,000 x *g* for 30 seconds. Discard the flow-through and place the column back into the Collection Tube. 8. Add **600 µL Wash Buffer G2** to the column. Centrifuge at 14,000 x *g* for 30 seconds. Discard the flow-through and place the column back into the Collection Tube.
Drying	9. Centrifuge the column at 14,000 x *g* for 3 minutes to dry the membrane.
Elution	10. Transfer the **PrimeWay Genomic II Column** to a new 1.5 mL microcentrifuge tube. 11. Add **100 µL preheated Elution Buffer** to the centre of the column membrane. Incubate at room temperature for at least 3 minutes. 12. Centrifuge at 14,000 x *g* for 30 seconds to elute the DNA.

E) Protocol – Yeast

Reagents Supplied by User

- ✓ 10 mM EDTA, pH 8.0
- ✓ Sorbitol Buffer [1.2 M Sorbitol; 10 mM $CaCl_2$; 0.1 M Tris-HCl pH 7.5]
- ✓ β-mercaptoethanol (β-Me)
- ✓ Zymolyase

Preparation	I. Set water bath/ dry bath to 60 °C. II. Preheat the **Elution Buffer** at 60 °C. III. **Sorbitol Mix** preparation: • Add **2.4 µL β-Me** (not provided) to **1 mL Sorbitol Buffer** (not provided). • Dissolve **200 U Zymolyase** (not provided) in **600 µL Sorbitol Buffer containing β-Me**.
Sample	1. Transfer yeast liquid culture to a new 1.5 mL microcentrifuge tube. Harvest up to **30 mg cell pellet** by centrifuging at 16,000 x *g* for 2 minutes. Discard supernatant. 2. Wash the cell pellet by resuspending with **1 mL 10 mM EDTA** (not provided). 3. Centrifuge at 5,000 x *g* for 10 minutes. Discard supernatant.
Lysis	4. Resuspend the pellet with **600 µL Sorbitol Mix** (not provided). Using a thermomixer, shake the tube at 1,000 rpm, 37 °C for 30 minutes. 5. Centrifuge at 2,000 x *g* for 10 minutes and remove 500 µL supernatant. ***Note**: Remain ~100 µL of supernatant containing spheroplasts cells.* 6. Add **200 µL GL1 Buffer** and **20 µL Proteinase K Solution**. Vortex to mix.

Lysis	7. Incubate the reaction at 60 °C for 30 minutes with shaking at 1,000 rpm using thermomixer. 8. Add **200 µL GL2 Buffer**. Vortex to mix. 9. Add **4 µL of RNase A Solution**. Vortex to mix and incubate at room temperature for 5 minutes.
Binding	10. Add **200 µL absolute ethanol** (not provided). Vortex to mix immediately. 11. Place a **PrimeWay Genomic II Column** into a new Collection Tube. 12. Transfer **up to 750 µL lysate**, including the precipitate if any, to the **PrimeWay Genomic II Column**. Centrifuge at 14,000 x *g* for 1 minute. 13. Discard the Collection Tube and place the column into a new Collection Tube.
Washing	14. Add **400 µL Wash Buffer G1** to the column. Centrifuge at 14,000 x *g* for 30 seconds. Discard the flow-through and place the column back into the Collection Tube. 15. Add **600 µL Wash Buffer G2** to the column. Centrifuge at 14,000 x *g* for 30 seconds. Discard the flow-through and place the column back into the Collection Tube.
Drying	16. Centrifuge the column at 14,000 x *g* for 3 minutes to dry the membrane.

Elution	17. Transfer the **PrimeWay Genomic II Column** to a new 1.5 mL microcentrifuge tube. 18. Add **50 µL preheated Elution Buffer** to the centre of the column membrane. Incubate at room temperature for at least 3 minutes. 19. Centrifuge at 14,000 x *g* for 30 seconds to elute the DNA.

F) Protocol – Fungi from cut agar

Reagent Supplied by User

- ✓ Sorbitol Buffer [1.2 M Sorbitol; 10 mM $CaCl_2$; 0.1 M Tris-HCl pH 7.5]
- ✓ β-mercaptoethanol (β-Me)
- ✓ Zymolyase

Preparation	I. Set water bath/ dry bath to 60 °C. II. Preheat the **Elution Buffer** at 60 °C. III. **Sorbitol Mix** preparation: • Add **2.4 µL β-Me** (not provided) to **1 mL Sorbitol Buffer** (not provided). • Dissolve **200 U Zymolyase** (not provided) in **600 µL Sorbitol Buffer containing β-Me**.
Sample	1. Cut **fungi sample (0.5 cm x 0.5 cm)** with minimum of agar from agar plate. Transfer sample into a new 1.5 mL microcentrifuge tube.
Lysis	2. Add **600 µL Sorbitol Mix** (not provided). Using a thermomixer, shake the tube at 1,000 rpm, 37 °C for 30 minutes. 3. Short spin to bring down the liquid. Transfer everything except the agar piece to a new 1.5 mL microcentrifuge tube. 4. Centrifuge at 13,000 x *g* for 10 minutes. Remove supernatant. 5. Resuspend cell pellet with **200 µL GL1 Buffer**. 6. Add **20 µL Proteinase K Solution**. Incubate the reaction at 60 °C for 30 minutes with shaking at 1,000 rpm using thermomixer.

Lysis	7. Add **200 µL GL2 Buffer**. Vortex to mix. 8. Add **4 µL RNase A Solution**. Vortex to mix and incubate at room temperature for 5 minutes.
Binding	9. Add **200 µL absolute ethanol** (not provided). Vortex to mix immediately. 10. Place a **PrimeWay Genomic II Column** into a new Collection Tube. 11. Transfer **up to 750 µL lysate**, including the precipitate if any, to the **PrimeWay Genomic II Column**. Centrifuge at 14,000 x *g* for 1 minute. 12. Discard the Collection Tube and place the column into a new Collection Tube.
Washing	13. Add **400 µL Wash Buffer G1** to the column. Centrifuge at 14,000 x *g* for 30 seconds. Discard the flow-through and place the column back into the Collection Tube. 14. Add **600 µL Wash Buffer G2** to the column. Centrifuge at 14,000 x *g* for 30 seconds. Discard the flow-through and place the column back into the Collection Tube.
Drying	15. Centrifuge the column at 14,000 x *g* for 3 minutes to dry the membrane.

Elution	
	16. Transfer the **PrimeWay Genomic II Column** to a new 1.5 mL microcentrifuge tube. 17. Add **50 µL preheated Elution Buffer** to the centre of the column membrane. Incubate at room temperature for at least 3 minutes. 18. Centrifuge at 14,000 x *g* for 30 seconds to elute the DNA.

G) Protocol – Whole Blood/ Buffy Coat

Reagent Supplied by User

✓ [Optional] 1X Phosphate Buffered Saline (PBS)

Preparation	I. Set water bath/ dry bath to 60 °C. II. Preheat the **Elution Buffer** at 60 °C.
Sample	1. Collect whole blood in EDTA tube (not provided). Store and transport the tube at 4 °C. 2. Add **20 µL Proteinase K Solution** to a new 1.5 mL microcentrifuge tube. 3. Transfer **200 µL whole blood/ buffy coat** to the tube. Mix by pipetting. Incubate at 60 °C for 5 minutes. ***Note:*** *If the sample is less than 200 µL, top up the volume to 200 µL with PBS (not provided).*
Lysis	4. Add **200 µL GL2 Buffer**. Vortex to mix and incubate at 60 °C for 5 minutes. 5. Add **4 µL RNase A Solution**. Vortex to mix and incubate at room temperature for 5 minutes.
Binding	6. Add **200 µL absolute ethanol** (not provided). Vortex to mix immediately. 7. Place a **PrimeWay Genomic II Column** into a new Collection Tube.

Binding	8. Transfer **up to 750 µL lysate**, including the precipitate if any, to the **PrimeWay Genomic II Column**. Centrifuge at 14,000 x *g* for 1 minute. 9. Discard the Collection Tube and place the column into a new Collection Tube.
Washing	10. Add **400 µL Wash Buffer G1** to the column. Centrifuge at 14,000 x *g* for 30 seconds. Discard the flow-through and place the column back into the Collection Tube. 11. Add **600 µL Wash Buffer G2** to the column. Centrifuge at 14,000 x *g* for 30 seconds. Discard the flow-through and place the column back into the Collection Tube.
Drying	12. Centrifuge the column at 14,000 x *g* for 3 minutes to dry the membrane.
Elution	13. Transfer the **PrimeWay Genomic II Column** to a new 1.5 mL microcentrifuge tube. 14. Add **100 µL preheated Elution Buffer** to the centre of the column membrane. Incubate at room temperature for at least 3 minutes. 15. Centrifuge at 14,000 x *g* for 30 seconds to elute the DNA.

H) Protocol – Nucleated Blood

Reagent Supplied by User

✓ 1X Phosphate Buffered Saline (PBS)

Preparation	I. Set water bath/ dry bath to 60 °C. II. Preheat the **Elution Buffer** at 60 °C.
Sample	1. Add **90 µL PBS** (not provided) to a new 1.5 mL microcentrifuge tube. 2. Add **10 µL nucleated blood** to the tube. Mix by pipetting.
Lysis	3. Add **100 µL GL1 Buffer** and **20 µL Proteinase K Solution**. Incubate at 60 °C for 10 minutes. 4. Add **200 µL GL2 Buffer**. Vortex to mix. 5. Add **4 µL RNase A Solution**. Vortex to mix and incubate at room temperature for 5 minutes.
Binding	6. Add **200 µL absolute ethanol** (not provided). Vortex to mix immediately. 7. Place a **PrimeWay Genomic II Column** into a new Collection Tube. 8. Transfer **up to 750 µL lysate**, including the precipitate if any, to the **PrimeWay Genomic II Column**. Centrifuge at 14,000 x *g* for 1 minute. 9. Discard the Collection Tube and place the column into a new Collection Tube.

Washing	10. Add **400 µL Wash Buffer G1** to the column. Centrifuge at 14,000 x *g* for 30 seconds. Discard the flow-through and place the column back into the Collection Tube. 11. Add **600 µL Wash Buffer G2** to the column. Centrifuge at 14,000 x *g* for 30 seconds. Discard the flow-through and place the column back into the Collection Tube.
Drying	12. Centrifuge the column at 14,000 x *g* for 3 minutes to dry the membrane.
Elution	13. Transfer the **PrimeWay Genomic II Column** to a new 1.5 mL microcentrifuge tube. 14. Add **100 µL preheated Elution Buffer** to the centre of the column membrane. Incubate at room temperature for at least 3 minutes. 15. Centrifuge at 14,000 x *g* for 30 seconds to elute the DNA.

I) Protocol – Dried Blood Spot (Nucleocard)

Preparation	I. Set water bath/ dry bath to 60 °C. II. Preheat the **Elution Buffer** at 60 °C.
Sample	1. Cut **1 or 2 dried blood spots (15 – 30 mm^2 each)** and place it into a new 1.5 mL microcentrifuge tube.
Lysis	2. Add **400 µL GL1 Buffer** and **20 µL Proteinase K Solution**. Vortex to mix. 3. Incubate the reaction at 60 °C for 60 minutes with shaking at 1,000 rpm using thermomixer. 4. Add **400 µL GL2 Buffer**. Vortex to mix.
Binding	5. Add **400 µL absolute ethanol** (not provided). Vortex to mix immediately. 6. Place a **PrimeWay Genomic II Column** into a new Collection Tube. 7. Transfer **up to 750 µL lysate**, including the precipitate if any, to the **PrimeWay Genomic II Column**. Centrifuge at 14,000 x *g* for 1 minute. Discard flow through. 8. Repeat Step 7 until all the sample has been transferred to the **PrimeWay Genomic II Column**. 9. Discard the Collection Tube and place the column into a new Collection Tube.

Washing	10. Add **400 µL Wash Buffer G1** to the column. Centrifuge at 14,000 x *g* for 30 seconds. Discard the flow-through and place the column back into the Collection Tube. 11. Add **600 µL Wash Buffer G2** to the column. Centrifuge at 14,000 x *g* for 30 seconds. Discard the flow-through and place the column back into the Collection Tube.
Drying	12. Centrifuge the column at 14,000 x *g* for 3 minutes to dry the membrane.
Elution	13. Transfer the **PrimeWay Genomic II Column** to a new 1.5 mL microcentrifuge tube. 14. Add **50 µL preheated Elution Buffer** to the centre of the column membrane. Incubate at room temperature for at least 3 minutes. 15. Centrifuge at 14,000 x *g* for 30 seconds to elute the DNA.

J) Protocol – White Blood Cells (Leukocytes)

Reagent Supplied by User

- ✓ RBC Lysis Buffer
- ✓ 1X Phosphate Buffered Saline (PBS)

Preparation	I. Set water bath/ dry bath to 60 °C. II. Preheat the **Elution Buffer** at 60 °C.
Sample	1. Collect whole blood in EDTA tube (not provided). Store and transport the tube at 4 °C. 2. Transfer **up to 1 mL whole blood** into a new 2 mL microcentrifuge tube. 3. Add **1 mL RBC Lysis Buffer** (not provided). Invert the tube 10 times. Centrifuge at 700 x *g* for 5 minutes. 4. Remove 1 mL top layer of the supernatant by pipetting. ***Note**: Do not remove the middle and bottom layer which is the white blood cells and red blood cells respectively.* 5. Add **1 mL RBC Lysis Buffer** (not provided). Resuspend the pellet by pipetting 4 – 5 times. Centrifuge at 700 x *g* for 5 minutes. 6. Carefully remove 1 mL supernatant by aspirate from top via pipetting. Leave the remaining supernatant and cell pellet in the tube. 7. Repeat Step 5. Remove supernatant **completely**.

Sample	8. [For preservation] Resuspend cell pellet in 1 mL RNALater Solution (not provided). Prior to DNA extraction, centrifuge the sample at 16,000 x *g* for 5 minutes. Remove the preservation solution completely.
Lysis	9. Resuspend cell pellet with **200 µL PBS** (not provided). 10. Add **20 µL Proteinase K Solution**. Vortex to mix and incubate at 60 °C for 5 minutes. 11. Add **200 µL GL2 Buffer**. Vortex to mix and incubate at 60 °C for 10 minutes. Invert the tube every 5 minutes. 12. Add **4 µL RNase A Solution**. Vortex to mix and incubate at room temperature for 5 minutes.
Binding	13. Add **200 µL absolute ethanol** (not provided). Vortex to mix immediately. 14. Place a **PrimeWay Genomic II Column** into a new Collection Tube. 15. Transfer **up to 750 µL lysate**, including the precipitate if any, to the **PrimeWay Genomic II Column**. Centrifuge at 14,000 x *g* for 1 minute. 16. Discard the Collection Tube and place the column into a new Collection Tube.
Washing	17. Add **400 µL Wash Buffer G1** to the column. Centrifuge at 14,000 x *g* for 30 seconds. Discard the flow-through and place the column back into the Collection Tube. 18. Add **600 µL Wash Buffer G2** to the column. Centrifuge at 14,000 x *g* for 30 seconds. Discard the flow-through and place the column back into the Collection Tube.

Drying	19. Centrifuge the column at 14,000 x *g* for 3 minutes to dry the membrane.
Elution	20. Transfer the **PrimeWay Genomic II Column** to a new 1.5 mL microcentrifuge tube. 21. Add **100 µL preheated Elution Buffer** to the centre of the column membrane. Incubate at room temperature for at least 3 minutes. 22. Centrifuge at 14,000 x *g* for 30 seconds to elute the DNA.

K) Protocol – Insects

Preparation	I. Set water bath/ dry bath to 60 °C. II. Preheat the **Elution Buffer** at 60 °C.
Sample	1. Grind insect to fine powder with liquid nitrogen using mortar and pestle. *Refer page 5 for details of Sample Disruption.* 2. Transfer up to **50 mg of insect powder** to a new 1.5 mL microcentrifuge tube.
Lysis	3. Add **200 µL GL1 Buffer** and **20 µL Proteinase K Solution**. Vortex to mix. 4. Incubate the sample at 60 °C for 1 to 3 hours. Invert the tube occasionally. 5. Centrifuge at 14,000 x *g* for 2 minutes to pellet insoluble debris. Transfer the supernatant to a new 1.5 mL microcentrifuge tube. 6. Add **200 µL GL2 Buffer**. Vortex to mix. 7. Add **4 µL RNase A Solution**. Vortex to mix and incubate at room temperature for 5 minutes.
Binding	8. Add **200 µL absolute ethanol** (not provided). Vortex to mix immediately. 9. Place a **PrimeWay Genomic II Column** into a new Collection Tube.

Binding	10. Transfer **up to 750 µL lysate**, including the precipitate if any, to the **PrimeWay Genomic II Column**. Centrifuge at 14,000 x *g* for 1 minute. 11. Discard the Collection Tube and place the column into a new Collection Tube.
Washing	12. Add **400 µL Wash Buffer G1** to the column. Centrifuge at 14,000 x *g* for 30 seconds. Discard the flow-through and place the column back into the Collection Tube. 13. Add **600 µL Wash Buffer G2** to the column. Centrifuge at 14,000 x *g* for 30 seconds. Discard the flow-through and place the column back into the Collection Tube.
Drying	14. Centrifuge the column at 14,000 x *g* for 3 minutes to dry the membrane.
Elution	15. Transfer the **PrimeWay Genomic II Column** to a new 1.5 mL microcentrifuge tube. 16. Add **100 µL preheated Elution Buffer** to the centre of the column membrane. Incubate at room temperature for at least 3 minutes. 17. Centrifuge at 14,000 x *g* for 30 seconds to elute the DNA.

L) Protocol – Buccal Swab

Reagent Supplied by User

✓ 1X Phosphate Buffered Saline (PBS)

Preparation	I. Set water bath/ dry bath to 60 °C. II. Preheat the **Elution Buffer** at 60 °C.
Sample	1. Firmly scrape the inner cheek several times using buccal swab. ***Note**: Do not consume any food or drink 30 minutes prior to sample collection.* 2. Air dry the swab. 3. Transfer **1 dry swab** to a new 2 mL microcentrifuge tube.
Lysis	4. Add **500 µL PBS** (not provided). Vortex to mix. 5. Add **20 µL Proteinase K Solution**. Incubate at 60 °C for 15 minutes. 6. Transfer **~400 µL lysate** to a new 1.5 mL microcentrifuge tube. Discard the tube with swab. 7. Add **400 µL GL2 Buffer**. Vortex to mix.
Binding	8. Add **400 µL absolute ethanol** (not provided). Vortex to mix immediately. 9. Place a **PrimeWay Genomic II Column** into a new Collection Tube. 10. Transfer **up to 750 µL lysate**, including the precipitate if any, to the **PrimeWay Genomic II Column**. Centrifuge at 14,000 x *g* for 1 minute. Discard flow through.

Binding	11. Repeat Step 10 until all the sample has been transferred to the **PrimeWay Genomic II Column**. 12. Discard the Collection Tube and place the column into a new Collection Tube.
Washing	13. Add **400 µL Wash Buffer G1** to the column. Centrifuge at 14,000 x *g* for 30 seconds. Discard the flow-through and place the column back into the Collection Tube. 14. Add **600 µL Wash Buffer G2** to the column. Centrifuge at 14,000 x *g* for 30 seconds. Discard the flow-through and place the column back into the Collection Tube.
Drying	15. Centrifuge the column at 14,000 x *g* for 3 minutes to dry the membrane.
Elution	16. Transfer the **PrimeWay Genomic II Column** to a new 1.5 mL microcentrifuge tube. 17. Add **50 µL preheated Elution Buffer** to the centre of the column membrane. Incubate at room temperature for at least 3 minutes. 18. Centrifuge at 14,000 x *g* for 30 seconds to elute the DNA.

M) Protocol – Saliva

Reagent Supplied by User

✓ 1X Phosphate Buffered Saline (PBS)

Preparation	I. Set water bath/ dry bath to 60 °C. II. Preheat the **Elution Buffer** at 60 °C.
Sample	1. Collect at least 1 mL saliva in a new 50 mL centrifuge tube. 2. Transfer **1 mL saliva** to a new 15 mL centrifuge tube. Add **5 mL PBS** (not provided). Vortex to mix vigorously. 3. Centrifuge at 2,000 x *g* for 5 minutes at room temperature to pellet cells. Immediately, decant the supernatant. 4. Resuspend pellet with **200 µL PBS** (not provided). Transfer the sample to a new 1.5 mL microcentrifuge tube.
Lysis	5. Add **20 µL Proteinase K Solution**. Incubate at 60 °C for 5 minutes. 6. Add **200 µL GL2 Buffer**. Invert tube 10 times to mix. 7. Incubate at 60 °C for 10 minutes. Invert the tube every 5 minutes. 8. Add **4 µL RNase A Solution**. Vortex to mix and incubate at room temperature for 5 minutes.
Binding	9. Add **200 µL absolute ethanol** (not provided). Invert tube 10 times to mix immediately. 10. Place a **PrimeWay Genomic II Column** into a new Collection Tube.

Binding	11. Transfer **up to 750 µL lysate**, including the precipitate if any, to the **PrimeWay Genomic II Column**. Centrifuge at 14,000 x *g* for 1 minute. 12. Discard the Collection Tube and place the column into a new Collection Tube.
Washing	13. Add **400 µL Wash Buffer G1** to the column. Centrifuge at 14,000 x *g* for 30 seconds. Discard the flow-through and place the column back into the Collection Tube. 14. Add **600 µL Wash Buffer G2** to the column. Centrifuge at 14,000 x *g* for 30 seconds. Discard the flow-through and place the column back into the Collection Tube.
Drying	15. Centrifuge the column at 14,000 x *g* for 3 minutes to dry the membrane.
Elution	16. Transfer the **PrimeWay Genomic II Column** to a new 1.5 mL microcentrifuge tube. 17. Add **100 µL preheated Elution Buffer** to the centre of the column membrane. Incubate at room temperature for at least 3 minutes. 18. Centrifuge at 14,000 x *g* for 30 seconds to elute the DNA.

N) Protocol – Alcohol-fixed Tissue

Reagent Supplied by User

- 1X Phosphate Buffered Saline (PBS)

Preparation	I. Set water bath/ dry bath to 60 °C. II. Preheat the **Elution Buffer** at 60 °C.
Sample	1. Transfer up to **25 mg alcohol-fixed tissue** to a new 1.5 mL microcentrifuge tube. 2. Add **1 mL PBS** to wash tissue. Discard the liquid completely by pipetting. 3. Repeat Step 2.
Lysis	4. Add **200 µL GL1 Buffer** and **20 µL Proteinase K Solution**. Vortex to mix. 5. Incubate at 60 °C for 3 hours/ overnight. Invert tube occasionally. 6. Centrifuge at 14,000 x *g* for 2 minutes to pellet insoluble debris. 7. Transfer the supernatant to a new 1.5 mL microcentrifuge tube. 8. Add **200 µL GL2 Buffer**. Vortex to mix.
Binding	9. Add **200 µL absolute ethanol** (not provided). Vortex to mix immediately. 10. Place a **PrimeWay Genomic II Column** into a new Collection Tube.

Binding	11. Transfer **up to 750 µL lysate**, including the precipitate if any, to the **PrimeWay Genomic II Column**. Centrifuge at 14,000 x *g* for 1 minute. 12. Discard the Collection Tube and place the column into a new Collection Tube.
Washing	13. Add **400 µL Wash Buffer G1** to the column. Centrifuge at 14,000 x *g* for 30 seconds. Discard the flow-through and place the column back into the Collection Tube. 14. Add **600 µL Wash Buffer G2** to the column. Centrifuge at 14,000 x *g* for 30 seconds. Discard the flow-through and place the column back into the Collection Tube.
Drying	15. Centrifuge the column at 14,000 x *g* for 3 minutes to dry the membrane.
Elution	16. Transfer the **PrimeWay Genomic II Column** to a new 1.5 mL microcentrifuge tube. 17. Add **100 µL preheated Elution Buffer** to the centre of the column membrane. Incubate at room temperature for at least 3 minutes. 18. Centrifuge at 14,000 x *g* for 30 seconds to elute the DNA.

O) Protocol – Sperm

Reagent Supplied by User

- ✓ Sperm Lysis Buffer [20 mM Tris-HCl, pH 8.0; 20 mM EDTA, pH 8.0; 200 mM NaCl; 4% SDS]
- ✓ 1 M Dithiothreitol (DTT) Solution
  [Dissolve 1.5 g DTT in 8 mL sterile water, top up to 10 mL. Make into aliquots, wrapped in aluminium foil and store in dark environment at -20 °C]

Preparation	I. Set water bath/ dry bath to 60 °C. II. Preheat the **Elution Buffer** at 60 °C. III. **Sperm Lysis Buffer Mix** preparation: Transfer **900 µL Sperm Lysis Buffer** (not provided) into a new 1.5 mL microcentrifuge tube. Add **80 µL DTT Solution** (not provided) and **20 µL Proteinase K Solution** immediately before use. Vortex to mix.
Lysis	1. Transfer **100 µL sperm sample** and **100 µL Sperm Lysis Buffer Mix** to a new 1.5 mL microcentrifuge tube. Invert to mix. Incubate at 60 °C for 1 hour. Invert the tube occasionally. 2. Add **200 µL GL2 Buffer** and **4 µL RNase A Solution**. Vortex to mix and incubate at room temperature for 5 minutes.
Binding	3. Add **200 µL absolute ethanol** (not provided). Vortex to mix immediately. 4. Place a **PrimeWay Genomic II Column** into a new Collection Tube. 5. Transfer **up to 750 µL lysate**, including the precipitate if any, to the **PrimeWay Genomic II Column**. Centrifuge at 14,000 x *g* for 1 minute.

Binding	6. Discard the Collection Tube and place the column into a new Collection Tube.
Washing	7. Add **400 µL Wash Buffer G1** to the column. Centrifuge at 14,000 x *g* for 30 seconds. Discard the flow-through and place the column back into the Collection Tube. 8. Add **600 µL Wash Buffer G2** to the column. Centrifuge at 14,000 x *g* for 30 seconds. Discard the flow-through and place the column back into the Collection Tube.
Drying	9. Centrifuge the column at 14,000 x *g* for 3 minutes to dry the membrane.
Elution	10. Transfer the **PrimeWay Genomic II Column** to a new 1.5 mL microcentrifuge tube. 11. Add **50 µL preheated Elution Buffer** to the centre of the column membrane. Incubate at room temperature for at least 3 minutes. 12. Centrifuge at 14,000 x *g* for 30 seconds to elute the DNA. 13. [Optional] For maximum recovery, repeat Step 11 and 12 with a new 1.5 mL microcentrifuge tube.

P) Protocol – Feathers/ Nails/ Hair

Reagent Supplied by User

✓ 1 M Dithiothreitol (DTT) Solution
[Dissolve 1.5 g DTT in 8 mL sterile water, top up to 10 mL. Make into aliquots, wrapped in aluminium foil and store in dark environment at -20 °C]

Preparation	I. Set water bath/ dry bath to 60 °C. II. Preheat the **Elution Buffer** at 60 °C.
Sample	**1A)** Feather i) Cut **2 – 5 quills' end** (~1 cm). Trim off excess barb and transfer to a new 1.5 mL microcentrifuge tube. **OR** **1B)** Nail i) Cut nails into small pieces. Transfer **10 – 25 mg nails** to a new 1.5 mL microcentrifuge tube. **OR** **1C)** Hair i) Cut **1 – 10 hair follicles** (0.5 – 1 cm from base of hair strand) and transfer into a new 1.5 mL microcentrifuge tube.
Lysis	2. Add **300 µL GL1 Buffer, 20 µL Proteinase K Solution** and **20 µL DTT Solution** (not provided). Vortex to mix. 3. Incubate at 60 °C for 1 – 3 hours/ overnight. Invert the tube occasionally. 4. Centrifuge at 14,000 x *g* for 2 minutes to pellet insoluble debris. Transfer the supernatant to a new 1.5 mL microcentrifuge tube.

Lysis	5. Add **300 µL GL2 Buffer**. Vortex to mix.
Binding	6. Add **300 µL absolute ethanol** (not provided). Vortex to mix immediately. 7. Place a **PrimeWay Genomic II Column** into a new Collection Tube. 8. Transfer **up to 750 µL lysate**, including the precipitate if any, to the **PrimeWay Genomic II Column**. Centrifuge at 14,000 x *g* for 1 minute. Discard flow through. 9. Repeat Step 8 until all sample has been transferred to the **PrimeWay Genomic II Column**. 10. Discard the Collection Tube and place the column into a new Collection Tube.
Washing	11. Add **400 µL Wash Buffer G1** to the column. Centrifuge at 14,000 x *g* for 30 seconds. Discard the flow-through and place the column back into the Collection Tube. 12. Add **600 µL Wash Buffer G2** to the column. Centrifuge at 14,000 x *g* for 30 seconds. Discard the flow-through and place the column back into the Collection Tube.
Drying	13. Centrifuge the column at 14,000 x *g* for 3 minutes to dry the membrane.
Elution	14. Transfer the **PrimeWay Genomic II Column** to a new 1.5 mL microcentrifuge tube. 15. Add **50 µL preheated Elution Buffer** to the centre of the column membrane. Incubate at room temperature for at least 3 minutes. 16. Centrifuge at 14,000 x *g* for 30 seconds to elute the DNA.

Troubleshooting Guidelines

Problems	Possible Reason	Recommended Action
Low DNA yield	Poor homogenisation of sample	Refer to page 5 “Sample Homogenisation” for details on homogenisation methods. Ensure that the sample is fully submerged in GL1 Buffer with Proteinase K. Make sure to invert tube occasionally during incubation.
	Inappropriate buffer preparation	Ensure the preparation of Proteinase K, RNase A and Wash Buffer G2 is according to the protocol in “Before Start”, refer to page 5.
	DNA Elution	Ensure Elution Buffer is preheated to 60 °C before applying directly to the centre of membrane.
PrimeWay Genomic II Column is clogged	Too much sample materials	Do not exceed the maximum sample input as suggested. Reduce sample amount when necessary.
		Prior to adding GL2 Buffer, centrifuge at 14,000 x *g* for 2 minutes to pellet down insoluble debris. Transfer the supernatant to a new 1.5 mL microcentrifuge tube.
	Inefficient lysis	Ensure tissue samples are grinded into fine powder using mortar and pestle with liquid nitrogen.
		Proteinase K activity decreased. Make multiple aliquots of Proteinase K Solution for storage to prevent multiple freeze-thaw.
		Use alternative homogenisation method such as rotor stator homogeniser.
Poor DNA quality	Ethanol carryover	Ensure Drying Step is performed accordingly to ensure column membrane is dry. Repeat centrifugation if necessary.
	Sample degraded	Use fresh sample to perform extraction.
	Presence of RNA	Perform RNase treatment. During extraction, add 4 µL RNase A Solution after the addition of GL2 Buffer, vortex to mix and incubate at room temperature for 5 minutes.

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

Product Ordering Information

Protocol	Part Number	Product Description	Remarks
Cultured cells; Whole blood/ Buffy coat; Nucleated blood; Buccal swab; Saliva; Alcohol-fixed tissue	1st BASE BUF-2041-1x500ml	1X Phosphate Buffered Saline (PBS), Biotechnology Grade, 500mL	
Bacteria, Gram (+ve)	1st BASE K.BUF-9105-50ml	Bacterial Pre-Lysis Buffer, 50mL	Sufficient for up to 275 preps
	1st BASE K.RGT-9108-110mg	Lysozyme, 110 mg/vial	Sufficient for 25 preps
	1st BASE BUF-1053-100ml-pH8.0	0.5M EDTA Solution pH 8.0, Biotechnology Grade, 100mL	Dilute from 500 mM stock solution to 50 mM working solution before use.
Yeast	1st BASE BUF-1053-100ml-pH8.0	0.5M EDTA solution, pH 8.0, Biotechnology Grade, 100mL	Dilute from 500 mM stock solution to 10 mM working solution before use.
	Nacalai Tesque 32020-05	D-Glucitol, EP Grade, 500G (Sorbitol)	Sorbitol Buffer Recipe *Short product stability: 6 months at 4 °C due to the presence of sorbitol.
	1st BASE BUF-1416-1L-pH7.5	1M Tris-HCl, pH7.5, Biotechnology Grade, 1L	
	Nacalai Tesque 08894-25	Calcium Chloride, SP Grade for Molecular Biology, 500G	
	1st BASE K.RGT-9107-20000U	Zymolyase(R)-20T, 20000 U/g, 1g	Add into Sorbitol Buffer, sufficient for 100 preps
	Nacalai Tesque 21438-82	2-Mercaptoethanol, SP Grade for Molecular Biology, 25G	Add into Sorbitol Buffer, sufficient for 100 preps

Protocol	Part Number	Product Description	Remarks
Fungi from cut agar	Nacalai Tesque 32020-05	D-Glucitol, EP Grade, 500G (Sorbitol)	Sorbitol Buffer Recipe *Short product stability: 6 months at 4 °C due to the presence of sorbitol.
	1st BASE BUF-1416-1L-pH7.5	1M Tris-HCl, pH7.5, Biotechnology Grade, 1L	
	Nacalai Tesque 08894-25	Calcium Chloride, SP Grade for Molecular Biology, 500G	
	1st BASE K.RGT-9107-20000U	Zymolyase(R)-20T, 20000 U/g, 1g	Add into Sorbitol Buffer, sufficient for 100 preps
	Nacalai Tesque 21438-82	2-Mercaptoethanol, SP Grade for Molecular Biology, 25G	Add into Sorbitol Buffer, sufficient for 100 preps
White blood cells	1st BASE K.BUF-9101-100ml	RBC Lysis Buffer, 100mL	Sufficient for up to 30 preps
	1st BASE BUF-2041-1x500ml	1X Phosphate Buffered Saline (PBS), Biotechnology Grade, 500mL	
Sperm	1st BASE K.BUF-9106-50ml	Sperm Lysis Buffer	Sufficient for up to 50 preps
	Nacalai Tesque 14128-04	Dithiothreitol, SP Grade for Molecular Biology, 5G	
Feathers/ Nails/ Hair	Nacalai Tesque 14128-04	Dithiothreitol, SP Grade for Molecular Biology, 5G