1st BASE
YOUR RESEARCH, OUR BUSINESS

SUPPLEMENTARY PROTOCOL

Ver 2.0

KIT-9240

PrimeMag Blood DNA Extraction Kit

A supplementary protocol is an additional guide provided alongside the main protocol to assist users in applying the product to special or less common sample types, workflows, or conditions. It expands the usability of the main kit by offering validated methods for alternative applications while maintaining performance and quality standards. The support protocol does not replace the main protocol, but serves as supplementary guidance.





List of Supplementary Protocol for KIT-9240

1. **Optional Equipment:** Thermomixer
   Sample Type: Whole Blood

2. **Sample type:** Nucleated Blood (non-mammalian)
   (e.g. fish, frogs, birds, reptiles and etc.)

1. **Optional Equipment:** Thermomixer
Sample type: Whole Blood

Materials Supplied by Users

- Centrifuge with speed of 12,000 x g (Microfuge 16, Beckman Coulter)
- Thermomixer with temperature settings of 65 °C and shake at 1,000 rpm (HM100-Pro Thermo Mix, DLAB or equivalent)
- Magnetic Rack (KIT-MAG16A, 1st BASE)
- Vortex mixer with moderate speed set to setting 5 and low speed set to setting 1 (Vortex-Genie 2, Scientific Industries)
- 2 mL microcentrifuge tubes
- 1.5 mL microcentrifuge tubes
- Isopropanol (IPA)
- Absolute ethanol (≥ 99.5%)

Protocol

Preparation

I. Set thermomixer to 65 °C.

II. Proteinase K Solution preparation:
Add indicated volume of **Proteinase K Buffer** to dissolve **Proteinase K**.

P/No	Proteinase K Buffer to be added
KIT-9240-10	500 µL
KIT-9240-96	2.5 mL

After reconstitution, store at – 20 °C.

III. Add **absolute ethanol (≥ 99.5%)** to **WBA Buffer** as following

P/No	WBA Buffer	Ethanol to be added
KIT-9240-10	9 mL	13.5 mL
KIT-9240-96	40 mL (each bottle)	60 mL (each bottle)

IV. Freshly prepare **80% ethanol** based on the number of preps required:

No of prep	Components	Volume	Total Volume
1	Absolute ethanol (> 99.5%)	640 µL	800 µL
	Nuclease-free water	160 µL	

V. Vortex the magnetic beads (**PrimeMag Beads A**) prior to use.

Sample	1. Transfer **200 µL whole blood** into a new 2 mL tube.
Lysis	2. Add **20 µL Proteinase K** and **200 µL PLB Lysis Buffer** into the sample tube. 3. **[Optional]** If RNA-free DNA is required, add **4 µL of RNase A** (100 mg/mL). 4. Vortex the sample for 10 seconds. Briefly centrifuge the tube. 5. Incubate at 65 °C for 10 minutes in a thermomixer at 1, 000 to 1, 500 rpm.
Binding	6. Add **360 µL Isopropanol (IPA)**, **120 µL BD2 Buffer** and **30 µL PrimeMag Beads A** sequentially to the sample tube. ***Note:*** *Vortex the PrimeMag Beads A for 30 seconds before use to ensure they remain fully suspended. A fresh premix of Isopropanol and BD2 Buffer can be prepared if required and must be used within 1 hour of preparation.* 7. Shake the mixture in a thermomixer at 1, 000 to 1, 500 rpm for 5 minutes at room temperature. Briefly centrifuge the tube. 8. Place the tube on the magnetic rack for 2 minutes, or until the **PrimeMag Beads A** have fully pelleted. 9. Carefully remove the supernatant by pipetting, taking care not to disturb the bead pellet. Discard the supernatant.

Washing	10. Add **800 µL WBA Buffer** (ethanol added) into the sample tube. 11. Shake the mixture in a thermomixer at 1, 000 to 1, 500 rpm for 1 minute at room temperature. Briefly centrifuge the tube. 12. Place the tube on the magnetic rack for 1 minute or until the **PrimeMag Beads A** separation has been completed. 13. Carefully remove the supernatant by pipetting, taking care not to disturb the bead pellet. Discard the supernatant. 14. Repeat step 10 – 13. 15. Add **800 µL 80% ethanol** into the sample tube. 16. Shake the mixture in a thermomixer at 1, 000 to 1, 500 rpm for 1 minute at room temperature. Briefly centrifuge the tube. 17. Place the tube on the magnetic rack for 1 minute or until the **PrimeMag Beads A** separation has been completed. 18. Carefully remove the supernatant by pipetting, taking care not to disturb the bead pellet. Discard the supernatant. 19. Air-dry the **PrimeMag Beads A** on magnetic rack at room temperature for 5 to 10 minutes. ***Note:*** *DO NOT over-dry the beads. Over-drying may reduce DNA recovery.*

Elution	20. Remove sample tube from the magnetic rack. Add **100 µL Elution Buffer** into the sample tube. 21. Shake the mixture in a thermomixer at 1, 000 to 1,500 rpm for 5 minutes at room temperature. 22. Place the tube on the magnetic rack for 2 minutes or until the **PrimeMag Beads A** separation has been completed. 23. Transfer the **supernatant** with purified DNA to a new 1.5 mL tube. ***Note:*** *Carefully transfer the supernatant without carrying over the magnetic beads.*



2. **Sample type: Sample type:** Nucleated Blood (non-mammalian) (e.g. fish, frogs, birds, reptiles and etc.)

Materials Supplied by Users

- Centrifuge with speed of 12,000 x g (Microfuge 16, Beckman Coulter)
- Thermomixer with temperature settings of 65 °C and shake at 1,000 rpm (HM100-Pro Thermo Mix, DLAB) or water bath for heating at 65 °C
- Magnetic Rack (KIT-MAG16A, 1st BASE)
- Vortex mixer with moderate speed set to setting 5 and low speed set to setting 1 (Vortex-Genie 2, Scientific Industries)
- 2 mL and 1.5 mL microcentrifuge tubes
- Isopropanol (IPA)
- Undenatured absolute ethanol (≥ 99.5%)
- (Optional: RNase A, 100 mg/ml)

Protocol

Preparation

I. Set thermomixer to 65 °C.

II. Proteinase K Solution preparation:
Add indicated volume of **Proteinase K Buffer** to dissolve **Proteinase K**.

P/No	Proteinase K Buffer to be added
KIT-9240-10	500 µL
KIT-9240-96	2.5 mL

After reconstitution, store at – 20 °C.

III. Add **absolute ethanol (≥ 99.5%)** to **WBA Buffer** as following

P/No	WBA Buffer	Ethanol to be added
KIT-9240-10	9 mL	13.5 mL
KIT-9240-96	40 mL (each bottle)	60 mL (each bottle)

IV. Freshly prepare **80% ethanol** based on the number of preps required:

No of prep	Components	Volume	Total Volume
1	Absolute ethanol (> 99.5%)	640 µL	800 µL
	Nuclease-free water	160 µL	

V. Vortex the magnetic beads (**PrimeMag Beads A**) prior to use.

Sample	1. Transfer **10 µL whole blood (with nucleated RBC)** into a new 2 mL tube. 2. Add **190 µL 1x PBS** (not provided) to the sample and mix by pipetting.
Lysis	3. Add **20 µL Proteinase K** and **200 µL PLB Lysis Buffer** into the sample tube. 4. **[Optional]** If RNA-free DNA is required, add **4 µL of RNase A** (100 mg/mL). 5. Vortex the sample for 10 seconds. Briefly centrifuge the tube. 6. Incubate at 65 °C for 10 minutes in a thermomixer at 1,000 rpm or in a water bath. If using a water bath, invert the tube 5 times, every 3 minutes during incubation.
Binding	7. Add **360 µL Isopropanol (IPA)**, **120 µL BD2 Buffer** and **30 µL PrimeMag Beads A** sequentially to the sample tube. ***Note:*** *Vortex the PrimeMag Beads A for 30 seconds before use to ensure they remain fully suspended. A fresh premix of Isopropanol and BD2 Buffer can be prepared if required and must be used within 1 hour of preparation.* 8. Vortex the mixture at moderate speed for 3 minutes and incubate at room temperature for 2 minutes. Briefly centrifuge the tube. 9. Place the tube on the magnetic rack for 2 minutes, or until the **PrimeMag Beads A** have fully pelleted. 10. Carefully remove the supernatant by pipetting, taking care not to disturb the bead pellet. Discard the supernatant.

Washing	11. Add **800 µL WBA Buffer** (ethanol added) into the sample tube. 12. Vortex at moderate speed for 1 minute and briefly centrifuge the tube. 13. Place the tube on the magnetic rack for 1 minute or until the **PrimeMag Beads A** separation has been completed. 14. Carefully remove the supernatant by pipetting, taking care not to disturb the bead pellet. Discard the supernatant. 15. Repeat step 11 – 13. 16. Add **800 µL 80% ethanol** into the sample tube. 17. Vortex at moderate speed for 1 minute and briefly centrifuge the tube. 18. Place the tube on the magnetic rack for 1 minute or until the **PrimeMag Beads A** separation has been completed. 19. Carefully remove the supernatant by pipetting, taking care not to disturb the bead pellet. Discard the supernatant.
Drying	20. Air-dry the **PrimeMag Beads A** on the magnetic rack at room temperature for 5 to 10 minutes. ***Note:*** *DO NOT over-dry the beads. Over-drying may reduce DNA recovery.*

Elution	21. Remove sample tube from the magnetic rack. Add **100 µL Elution Buffer** into the sample tube. 22. Vortex at the lowest speed for 3 minutes and incubate at room temperature for 2 minutes. 23. Place the tube on the magnetic rack for 2 minutes or until the **PrimeMag Beads A** separation has been completed. 24. Transfer the **supernatant** with purified DNA to a new 1.5 mL tube. ***Note:*** *Carefully transfer the supernatant without carrying over the magnetic beads.*

Product Ordering Information

Product Number	Product Description	Remarks
KIT-9240-10	PrimeMag Blood DNA Extraction Kit, 10 preps	Sufficient for 10 preps.
KIT-9240-96	PrimeMag Blood DNA Extraction Kit, 96 preps	Sufficient for 96 preps.
KIT-MAG16A	1st BASE Magnetic Rack (1.5ml/2.0ml), for 16 tubes	For 16 tubes processing.

Additional Buffers and Reagents Ordering Information

Protocol	Product Number	Product Description	Remarks
A. Whole blood B. Buffy coat C. Dried blood spot	(Optional) K.RGT-9110-1ml	RNase A Solution, 100mg/mL, 1mL	Sufficient for 250 preps
D. Cultured cells	K.RGT-9110-1ml	RNase A Solution, 100mg/mL, 1mL	Sufficient for 250 preps
	K.RGT-9105-50ml	Diluent Solution, 50ml	Sufficient for 250 preps
E. Buccal swab F. Saliva	(Optional) K.RGT-9110-1ml	RNase A Solution, 100mg/mL, 1mL	Sufficient for 250 preps
	BUF-2041-1x500ml	1X Phosphate Buffered Saline (PBS), Biotechnology Grade, 500ml	Sufficient for 450 preps



Document Revision History

Revision	Date of Revision	Description of Change
1.0	28-Sept-26	1. Initial Release
2.0	22-Oct-26	1. Added new sample type to the supplementary protocol: ➢ Sample type: Sample type: Nucleated Blood (non-mammalian) (e.g. fish, frogs, birds, reptiles and etc.)

