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SUPPLEMENTARY PROTOCOL

Ver 1.0

KIT-9021

PrimeWay Total RNA Extraction Kit

A support protocol is an extra guide provided with the main protocol to help users use the product with special or less common sample types, workflows, or conditions. It helps expand the use of the main kit by giving tested methods for other applications, while still ensuring good performance and quality. It doesn't replace the main protocol but offers extra help for different situations.

List of Supplementary Protocol for KIT-9021

1. Saliva
2. MRSA bacteria (Gram-positive)

1. Saliva

Materials Supplied by User

- ✓ 1.5 mL RNase-free microcentrifuge tube
- ✓ 50 mL centrifuge tube
- ✓ 20-gauge needle
- ✓ 1 mL sterile syringe
- ✓ RNA*later* solution
- ✓ Absolute ethanol
- ✓ 1X PBS
- ✓ β-ME (beta-mercaptoethanol)

Sample	1. Collect **at least 2 mL saliva** in a new 50 mL centrifuge tube. 2. Add **equal volume of RNA*later* solution** (2 mL if saliva collected is 2 mL). 3. Vortex vigorously to mix. 4. Incubate at 4 ˚C for overnight. ***Note:*** If RNA extraction is not performed directly, store the sample in -80 ˚C until further processing. 5. Thaw the sample on ice (if after stored at -80 oC). Add equal volume of ice cold 1X PBS (4 mL). Vortex to mix. 6. Centrifuge at maximum speed (6,000 x g) for 5 minutes at 4 oC. Immediately, decant the supernatant.
Lysis	7. Resuspend the pellet with **800 µL TR Buffer** and **8 µL β-ME** (not provided). ***Note:*** *If process large number of samples, user can prepare the TR Buffer with β-ME in large scale before start.*

Lysis	8. Homogenize the lysate using **20-gauge needle, fitted to 1 mL sterile syringe** (not provided). Passing the sample through the needle with at least 5 times or until a homogenous lysate is achieved. 9. Centrifuge at 16,000 x *g* for 3 minutes.
Filtration	10. Place a **PrimeWay Filter Column (Blue)** into a new Collection Tube. Transfer the supernatant in Step 9 into the **PrimeWay Filter Column** and centrifuge at 16,000 x *g* for 3 minutes. 11. Discard the **PrimeWay Filter Column**. **KEEP THE FLOW-THROUGH!**
Binding	12. Add **400 µL of absolute ethanol** (not provided) to the flow-through and mix well by pipetting. Do not centrifuge. 13. Place a **PrimeWay RNA Column (White)** into a new Collection Tube. 14. Transfer **up to 700 µL of the sample** to the **PrimeWay RNA Column**. Centrifuge at 12,000 x *g* for 1 minute. Discard the flow-through and place the column back into the Collection Tube. 15. Repeat step 14 until all the sample has been transferred to the **PrimeWay RNA Column**.

Washing

16. Add **700 µL Wash Buffer R1** to the **PrimeWay RNA Column**. Centrifuge at 12,000 x *g* for 1 minute. Discard the flow-through and place the column back into the Collection Tube.

17. [On-column DNase Treatment]
    i) Prepare DNase I Solution in a new 1.5 mL microcentrifuge tube (RNase-free) as below:

Components	1 Reaction
DNase I (2 U/µL)	5 µL
DNase I Buffer	45 µL
DNase I Solution (Total Volume)	**50 µL**

Mix gently by pipetting the solution. DO NOT Vortex.

    ii) Add **50 µL DNase I solution** into the CENTER of the column. Incubate the column for 15 minutes at room temperature.

18. Add **500 µL Wash Buffer R1** to the column. Centrifuge at 12,000 x *g* for 1 minute. Discard the flow-through and place the column back into the Collection Tube.
19. Add **500 µL Wash Buffer R2** to the column. Centrifuge at 12,000 x *g* for 1 minute. Discard the flow-through and place the column back into the Collection Tube.

20. Repeat step 19.

Drying

21. Centrifuge the column at 12,000 x g for 2 minutes to dry the membrane.

Elution	22. Transfer the **PrimeWay RNA Column** to a new 1.5 mL microcentrifuge tube. 23. Add **30 - 60 µL RNase-Free Water** to the center of the **PrimeWay RNA Column**. Incubate at room temperature for 2 minutes. 24. Centrifuge at 12,000 x *g* for 1 minute to elute the total RNA. 25. Store purified RNA at -80 ˚C.

2. MRSA bacteria (Gram-positive)

Materials Supplied by User

- ✓ 2 mg/mL Lysozyme [Dissolve 2 mg Lysozyme into 1 mL of 1X TE Buffer, freshly prepared]
- ✓ 70% ethanol

Sample	1. Retrieve sample of up to 100 mg cell pellet in RNAlater. 2. Remove RNAlater by centrifuge at 2,500 xg for 5 minutes.
Lysis	3. Add 100 µL freshly prepared TE (with 2 mg/mL lysozyme; not provided) to resuspend pellet by pipetting up and down. 4. Incubate at 37 °C for 10 minutes. 5. Add 700 µL TR + 7 µL β-ME (not provided) into sample tube. **6. Aliquot to a standing tube with beads (4-mm stainless steel beads) and bead beating with Disruptor Genie at the max speed for 5 minutes.** 7. Centrifuge at the max speed for 2 minutes. 8. Transfer lysate to a new 1.5 mL tube, use needle and syringe (not provided) to homogenize the sample for 10 times.

Filtration

9. Place a PrimeWay Filter Column (Blue) into a new Collection Tube. Transfer the homogenized lysate from Step 8 into the PrimeWay Filter Column and centrifuge at **12,000 x g for 3 minutes**.

10. Discard the PrimeWay Filter Column. **KEEP THE FLOW-THROUGH!**

Binding

11. Add 700 µL of 70% ethanol (not provided) to the flow through of each tube and mix well by pipetting. Do not centrifuge.

12. Place a PrimeWay RNA Column (White) into a new Collection Tube.

13. Transfer up to 700 µL of the sample to the PrimeWay RNA Column. Centrifuge at **12,000 x g for 3 minutes**. Discard the flow through and place the column back into the Collection Tube.

14. Repeat Step 14 until all the samples have been transferred to the PrimeWay RNA Column.

Washing

15. Add 700 µL Wash Buffer R1 to the PrimeWay RNA Column. Centrifuge at 12,000 x g for 1 minute. Discard the flow-through and place the column back into the Collection Tube.

16. [On-column DNase Treatment]
    i) Prepare DNase I Solution in a new 1.5 mL microcentrifuge tube (RNase-free) as below:

Components	1 Reaction
DNase I (2 U/µL)	5 µL
DNase I Buffer	45 µL
DNase I Solution (Total Volume)	**50 µL**

Mix gently by pipetting the solution. DO NOT Vortex.

    ii) Add 50 µL DNase I solution into the CENTRE of the column. Incubate the column for 15 minutes at room temperature.

Washing	17. Add 500 µL Wash Buffer R1 to the column. Centrifuge at 12,000 x g for 1 minute. Discard the flow-through and place the column back into the Collection Tube. 18. Add 500 µL Wash Buffer R2 to the column. Centrifuge at 12,000 x g for 1 minute. Discard the flow-through and place the column back into the Collection Tube. 19. Repeat step 19.
Drying	20. Centrifuge the column at 12,000 x g for 2 minutes to dry the membrane.
Elution	21. Transfer the PrimeWay RNA Column to a new 1.5 mL microcentrifuge tube. 22. Add **50 µL RNase-Free Water** to the centre of the PrimeWay RNA Column. Incubate at room temperature for 2 minutes. 23. Centrifuge at 12,000 x g for 1 minute to elute the total RNA. 24. Store purified RNA at −80 °C.

Agarose Gel Electrophoresis

Aliquots of 1 µl RNA were run on 1% TAE agarose gel at 100 V for 60 min.