MagGenome
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XpressRNA Cell Kit

Kit Components

Components	Storage Temperature
RDB	RT
RLB	RT
RPS	RT
RNA MagNa Mix	2-8°C
RWB1	RT
RWB2	RT
REB	RT
Proteinase K	2-8°C
Proteinase K dilution buffer	2-8°C
MagNa stand (optional)	RT

Specification:

Starting material	Fresh Human saliva - 200 µl
	Bacteria - OD ≤ 0.8
	Cultured cells - Up to $1x10^6$ cells
Elution volume	30-100 µl
Yield	Varies depending on sample type
Purity	260/280 ≥ 2.0

Equipment and Reagents to be supplied by user

- 96–100% ethanol
- RNase-free DNase (optional step)
- Sterile, RNase-free pipette tips
- Microcentrifuge (with rotor for 1.5 ml tubes)

IMPORTANT POINTS PRIOR TO START

- Set the water bath/heat block at 65℃.
- Reconstitute Proteinase K with Proteinase K dilution buffer and store at 2-8℃.
- Bring the RNA Magna Mix to room temperature before use.
- Add 96 - 100% Ethanol to RWB1 and RWB2 as indicated on the bottle.
- *To eliminate gDNA contamination from the final RNA eluate, perform DNase treatment (user supplied) as per user's lab protocol.*

Protocol

Sample Preparation

- Whole saliva

1. Take **200 µl fresh human saliva** in a sterile, RNase free 1.5 ml microcentrifuge tube.
2. Immediately proceed to step 9 (Lysate Preparation).

- Bacterial culture

3. Take **1ml bacterial culture (OD≤ 0.8)** in a sterile, RNase free 1.5 ml microcentrifuge tube and centrifuge at 10, 000 rpm for 5 minutes at RT. Discard the supernatant completely.
4. Resuspend the pellet completely in **200 µl RDB** by pipette mixing.
5. Immediately proceed to step 9 (Lysis).

- Cultured cells

6. Take **up to 1 x 10^6** cultured cells in a sterile, RNase free 1.5 ml microcentrifuge tube and centrifuge at 4,000 rpm for 5 minutes at RT. Discard the supernatant completely.
7. Resuspend the pellet completely in **200 µl RDB** by pipette mixing.
8. Immediately proceed to step 9 (Lysis).

Lysis

9. Add **400 µl RLB**. Vortex the contents for 60 seconds.
10. Add **10 µl Proteinase K** and vortex the tube for 30-60 seconds.
11. Incubate at 65 °C for 15 minutes.

Precipitation

12. Add **100 µl RPS** and vortex the contents for 30-60 seconds.
13. Centrifuge the tube at 14, 000 rpm for 3 minutes at RT.

Binding and Washing

Note: Vortex the RNA MagNa Mix to ensure complete dispersion of the particles.

14. Transfer the clear supernatant to a fresh, sterile RNase free 1.5 ml micro centrifuge tube and discard the pellet.
15. Add **600 µl RNA MagNa Mix** to the supernatant. Gently mix the contents by inverting the tube for 8-10 times.
16. Incubate at RT for 5 minutes.
17. Place the tube on MagNa Stand for 5 minutes at room temperature.
18. Carefully discard the supernatant without removing the tube from the MagNa Stand (make sure the pellet is not disturbed).
19. Add **1 ml RWB1** and remove the tube from MagNa Stand.
20. Resuspend the pellet by pipette mixing for 10-12 times (Ensure complete dispersion of particles).
21. Place the tube back on MagNa Stand. Keep until the solution becomes clear (30-60 seconds).
22. Discard the supernatant completely without removing the tube from MagNa Stand (Ensure the pellet is not disturbed).
23. Add **1 ml RWB2** and and gently invert the tube placed on MagNa Stand for 10-12 times to wash the pellet (Surface wash only).
24. Discard the supernatant completely without removing the tube from MagNa Stand.
25. Repeat the steps 23-24.
26. Air dry the pellet at RT for 10-15 minutes by keeping the tube on MagNa Stand.

Note: Do not over dry the pellet.

Elution

27. After drying, remove the tube from MagNa Stand.
28. Add **30-50 µl REB** and completely resuspend the pellet by pipette mixing for 10-15 times.
29. Incubate at RT for 2 minutes.
30. Place the tube on MagNa Stand for 5 minutes or until the solution appears clear (place the magnetic stand on ice).
31. Carefully transfer the supernatant containing RNA to a sterile RNase-free 1.5 ml micro centrifuge tube without removing the tube from MagNa Stand (make sure the pellet is not disturbed).
32. Discard the MagNa particles.

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Troubleshooting Guide

Observation	Possible causes	Suggested solution
Low RNA Yield or Poor Quality	Too much sample	Ensure to use the specified starting material to match the kit specifications
	Poor cell lysis	Ensure complete dispersion of sample in lysis buffer by vortex mixing
	Improper binding	After mixing with the MagNa Mix, the solution must be incubated at RT for 5 minutes and then placed on MagNaStand until the solution becomes clear.
	Ethanol is not added to wash buffers	Add 100% ethanol to wash buffers as suggested on the bottles.
	Incomplete elution	● Ensure complete dispersion of pellet in elution buffer. ● Increase incubation time for elution to 3-5 minutes.
High DNA contamination	Incomplete precipitation	Follow user manual for efficient DNA removal. If unsure, please repeat the protocol.
	Improper washing	Ensure to disperse the pellet completely in Wash Buffer-I
	Too much sample	Ensure to use the specified starting material to match the kit specifications
Degraded RNA	Saliva sample is degraded	Collect the saliva and transfer the collection tube on to ice as quickly as possible
	RNase contamination	After elution, the RNA eluate must be properly stored on ice for short term storage or at -80°C for long term storage.
		Although all XpressRNA kit buffers were tested and are guaranteed RNase-free, RNase can be introduced during use. Be certain not to introduce RNase during isolation process.
	Deviation from standard protocol for working with RNA	Refer to general guidelines for working with RNA
Unusual yield recovery	RNA concentration is too low for quantitative analysis	Elute with low volume (30 µl) of elution buffer for concentrated RNA
		Increase the amount of starting material (within kit specifications)

For queries, please write to support@maggenome.com

For complaints, please write to qc@maggenome.com