XpressRNA Viral Kit

Protocol for isolation of Total Viral RNA from oral and nasopharyngeal swabs collected in Viral Transport Medium (VTM) or Molecular Transport Medium (MTM).

Process Flow

Kit Contents

Components	Storage Conditions	Shipping Conditions
Viral Lysis Buffer	RT	RT
Viral MagNa Mix	2 - 8 °C	RT
Viral Wash Buffer	RT	RT
Viral Elution Buffer	RT	RT
MagNa Stand (optional)	RT	RT
Carrier RNA	-20°C	RT
Carrier RNA dilution buffer	-20°C	RT

*RT denotes 15 - 25°C.

Materials not provided with the kit

1. 100% Ethanol to Wash Buffers as indicated on the bottle.
2. Water bath/heat block at 56°C.
3. Reconstitute Carrier RNA with Carrier RNA dilution buffer and store at -20°C (Stock concentration: 1 mg/ml).

Important

Pay attention to standard lab practices and safety information before beginning the procedure. For more information, refer the appropriate Material Safety Data Sheet (MSDS) available from the product supplier or download from our website http://www.maggenome.com/

Technical Support

For any product related queries please write to us on info@maggenome.com, sales@maggenome.com, support@maggenome.com.

Protocol

Lysate Preparation	1. Take **200 µl of VTM/fluid sample** in a 1.5 ml tube. 2. Add **100 µl of Viral Lysis Buffer** and pipette mix the contents thoroughly. 3. Add **5 µl of Carrier RNA** and pipette mix. 4. Vortex the tube for 30 seconds**.** 5. Briefly spin the tube to bring down the residues from the inside of the lid. 6. Incubate for 10 minutes at 56°C.

RNA Binding	*(Note: Vortex the Viral MagNa Mix to ensure complete dispersion of the particles.)* 7. Add 125 µl of Viral MagNa Mix to the lysate and gently invert the tube 10 - 15 times to mix properly. Do not vortex. 8. Incubate at RT for 5 minutes. 9. Place the tube on Magna Stand until the solution becomes clear. 10. Carefully discard the supernatant completely without removing the tube from the MagNa Stand. Ensure the magnetic nanoparticles are not disturbed.

RNA Washing	11. Add **250 µl of Viral Wash Buffer** and gently invert the tube for 10 - 15 times without removing the tube from MagNa Stand *(surface wash only).* 12. Discard the supernatant without removing the tube from MagNa Stand. 13. Repeat the steps **11 - 12.** 14. Air dry the magnetic nanoparticles without removing the tube from the MagNa Stand at RT for 2 minutes without over drying them.

RNA Elution	15. After drying, remove the tube from the MagNa Stand. 16. Add **30 µl of Viral Elution buffer** and completely resuspend the magnetic nanoparticles by pipette mixing *(10 – 15 times).* 17. Incubate at 56°C for 2 minutes with intermittent tapping. *(Note: Briefly spin the tube for 5 seconds before placing on the MagNa stand.)* 18. Place the tube on MagNa Stand for 5 minutes or until the solution appears clear *(place the magnetic stand on ice).* 19. Carefully transfer the supernatant containing RNA to a nuclease free 1.5 ml tube without removing the tube from MagNa Stand. Ensure the magnetic nanoparticles are not disturbed. 20. Discard the magnetic nanoparticles in the appropriate hazard container.

Troubleshooting Guide

Observation	Possible causes	Suggested Solution
Degraded RNA	RNase contamination	XpressRNA kit buffers are tested and guaranteed RNase-free, although RNase can be introduced during use. Be certain not to introduce RNase during storage and handling.
	Carrier RNA storage	Ensure that carrier RNA and dilution buffer are stored properly at the recommended storage temperature.
Low RNA yield or Poor Quality	Incomplete Lysis	Make sure that the incubation temperature and time for lysis is followed as per the protocol.
	MagNa Mix was improperly handled	Ensure proper dispersion of nanoparticles by vortexing the MagNa Mix prior to use.
	Magnetic nanoparticle loss during binding or washing steps	Carefully remove the supernatant from the tube without removing the tube from the MagNa Stand and without disturbing the magnetic nanoparticles.
	Ethanol is not added to wash buffer	Add 100% ethanol to wash buffer before use as indicated on the bottles.
	Improper elution	Completely resuspend the magnetic nanoparticles in elution buffer before incubation at 56°C for elution.
Poor performance of extracted RNA in downstream applications	Ethanol carryover	Air dry the magnetic nanoparticles after the washing steps to remove ethanol traces completely, but do not over dry the pellet.