XpressDNA Saliva Kit (Stored Saliva DNA extraction)

Protocol for isolation of high quality total genomic DNA from stored saliva samples.

Process Flow

Kit Contents

Components	Storage Conditions	Shipping Conditions
Proteinase K	2 - 8 °C	RT
Proteinase K Buffer	2 - 8 °C	RT
RNase A	2 - 8 °C	RT
Saliva MagNa Mix	RT	RT
Saliva Wash Buffer 1	RT	RT
Saliva Wash Buffer 2	RT	RT
Saliva Elution Buffer	RT	RT
MagNa Stand (optional)	RT	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 Proteinase K with Proteinase K Buffer and store at 2 – 8°C.

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

Saliva Lysate Preparation	1. Take **1 ml of sample (Saliva stored in collection buffer)** into a sterile 1.5 ml tube *(Note: For collection of saliva and storage, refer the collection kit instructions as provided by the Manufacturer).* 2. Add **25 µl of Proteinase K** and pipette mix the contents thoroughly. 3. Incubate at 56˚C for 30 minutes. 4. Add **10 µl of RNase A** and perform a short vortex for 30 seconds. 5. Incubate at RT for 15 minutes. 6. Centrifuge at 14,000 rpm for 5 minutes at RT. 7. Transfer the supernatant to a fresh 1.5 ml tube.

DNA Binding	*(Vortex Saliva MagNa Mix thoroughly before the next step)* 8. To the supernatant, add **400 µl of Saliva MagNa Mix**. Gently invert mix the contents 10 - 12 times. Do not vortex. Incubate at RT for 5 minutes. 9. Place the tube on MagNa Stand for 5 minutes. 10. Carefully discard the supernatant without removing the tube from MagNa Stand Ensure the magnetic nanoparticles are not disturbed.

DNA Washing	11. Add **1 ml of Saliva Wash Buffer 1**, remove the tube from MagNa Stand and resuspend the pellet by pipette mixing thoroughly to ensure complete dispersal of the particles. Do not vortex. 12. Place the tube back on MagNa Stand for 30 - 60 seconds until the solution becomes clear. 13. Carefully discard the supernatant without removing the tube from MagNa Stand. Ensure the magnetic nanoparticles are not disturbed. 14. Add **1 ml of Saliva Wash Buffer 2** and gently invert mix 5 - 6 times without removing the tube from MagNa Stand to wash the pellet *(surface wash only).* 15. Discard the supernatant removing the tube from MagNa Stand. 16. Repeat steps **14 - 15.** 17. Air dry the magnetic nanoparticles without removing the tube from MagNa Stand at room temperature for 10 - 15 minutes. Avoid over drying.

DNA Elution	18. After drying, remove the tube from MagNa Stand. 19. Add **50 µl of Elution Buffer** and resuspend the magnetic nanoparticles by pipette mixing thoroughly. 20. Incubate at 56˚C for 10 minutes with intermittent tapping. 21. Place the tube on MagNa Stand for 5 minutes or until the solution becomes clear. 22. Carefully transfer the supernatant containing DNA to a sterile 1.5 ml tube, without

	removing the tube from MagNa Stand. Ensure the magnetic nanoparticles are not disturbed. 23. Discard the magnetic nanoparticles in appropriate hazard container.

Note: *In the elution step, if the Magnetic nanoparticles take more than 10 minutes for clearing, spin the tubes at 14,000 rpm for 5 minutes, place on MagNa Stand until solution clears and then collect the supernatant with pure DNA.*

Troubleshooting Guide

Problem	Possible causes	Suggested Solutions
Low DNA yield	Incomplete lysis	Use suggested amount of Proteinase K for the specified time.
	Incorrect reagent volumes were used	Use the exact volumes of reagents mentioned in the protocol.
	MagNa Mix was improperly handled	Resuspend the MagNa Mix by vortexing prior to use.
	Sample was improperly handled	Resuspend the collected saliva sample using pipette for uniform dispersion.
	MagNa Mix was disturbed or lost during binding or washing steps.	Carefully remove the supernatant from the tube without removing the tube from the MagNa Stand without disturbing the Magnetic nanoparticles.
	Improper elution	Completely resuspend the Magnetic nanoparticles in elution buffer before incubation at 56°C for elution.
	Ethanol is not added to wash buffers	Add 100% ethanol to wash buffers prior to use as mentioned on the bottles.
Poor performance of extracted DNA in downstream applications	Ethanol carryover	Air dry the Magnetic nanoparticles properly after washing steps, to remove the ethanol completely, but do not over dry the pellet.
	Salt carryover	Ensure that the correct amount of ethanol added to the Wash Buffers and two wash steps are performed with Wash Buffer 2.