## XpressDNA Saliva Kit (For fresh sample)

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

### Process Flow

### Kit Content

| Components | Storage Conditions | Shipping Conditions |
|---|---|---|
| Saliva Stabilization Buffer | RT | RT |
| Saliva Lysis Buffer | RT | RT |
| 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.

## Sample Collection

Collect fresh saliva samples from healthy subjects in a sterile microcentrifuge tube or appropriate saliva collection vials. Ensure subjects refrain from eating or drinking up to 30 minutes prior to sample collection. Subjects must wash mouth thoroughly with water 10 minutes prior to sample collection.

## Protocol

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| **Saliva Lysate Preparation** | 1. To a sterile 1.5 ml tube, **add 0.5 - 1.0 ml of saliva sample.**<br>2. Add **equal volume of Saliva Stabilization Buffer** and pipette mix the contents thoroughly.<br>3. Incubate at RT for 5 minutes.<br>4. Centrifuge at 14,000 rpm at RT for 5 minutes.<br>5. Carefully discard the supernatant and add **250 µl of Saliva Lysis Buffer** to the pellet.<br>6. Resuspend the pellet in the buffer by pipette mixing thoroughly.<br>7. Add **10 µl of Proteinase K** and perform a short vortex mix.<br>8. Incubate at 56°C for 15 minutes.<br>9. Add **10 µl of RNase A** and perform a short vortex mix<br>10. Incubate at RT for 15 minutes.<br>11. Centrifuge at 14,000 rpm at RT for 5 minutes.<br>12. Transfer the supernatant to a fresh 1.5 ml tube. |

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| **DNA Binding** | *(Vortex MagNa mix thoroughly before the next step)*<br>13. To the supernatant, add **175 µl of Saliva MagNa Mix**. Gently invert mix the contents 10 - 12 times. Do not vortex. Incubate at RT for 5 minutes.<br>14. Place the tube on a MagNa Stand for 5 minutes.<br>15. Carefully discard the supernatant without removing the tube from the MagNa Stand. Ensure the magnetic nanoparticles are not disturbed. |

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| **DNA Washing** | 16. Add **200 µl of Saliva Wash Buffer 1**, remove the tube from the MagNa Stand and resuspend the pellets by pipette mixing thoroughly to ensure complete dispersal of the particles. Do not vortex.<br>17. Place the tube back on the MagNa Stand for 30 - 60 seconds until the solution becomes clear.<br>18. Carefully discard the supernatant without removing the tube from the MagNa Stand. Ensure the magnetic nanoparticles are not disturbed.<br>19. Add **200 µl of Saliva Wash Buffer 2** and gently invert 5 - 6 times without removing the tube from the MagNa Stand to wash the pellet ***(surface wash only).***<br>20. Discard the supernatant without removing the tube from the MagNa Stand.<br>21. Repeat steps **19 - 20.**<br>22. Air dry the magnetic nanoparticles without removing the tube from the MagNa Stand for 10 - 15 minutes. Avoid over drying. |

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| **DNA Elution** | 16. After drying, remove the tube from the MagNa Stand.<br>17. Add **50 µl of Elution Buffer** and resuspend the magnetic nanoparticles by pipette mixing thoroughly.<br>18. Incubate at 56°C for 10 minutes with intermittent tapping.<br>19. Place the tube on the MagNa Stand for 5 minutes or until the solution becomes clear.<br>20. Carefully transfer the supernatant containing DNA to a sterile 1.5 ml tube, without removing the tube from the MagNa Stand. Ensure the magnetic nanoparticles are not disturbed.<br>21. 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.*

***Note:*** *Sputum samples can also be processed with the same protocol*

## Troubleshooting Guide

| Observation | Possible causes | Suggested Solution |
|---|---|---|
| **Low DNA yield or Poor Quality** | Incomplete lysis | Use suggested amount of Proteinase K for the specified time. |
| | 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. |
| | Age of sample used | Use fresh saliva sample or sample stored at -20ºC (< two weeks old). |
| | 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 56ºC 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. |