BUCCAL DNA EXTRACTION – MINI KIT

Materials needed prior to start

- Add 96 - 100% Ethanol to Wash Buffers as indicated on the bottle.
- Set the water bath/heat block at 56°C.
- Reconstitute Proteinase K with Proteinase K Buffer and store at 2 - 8°C.

PROTOCOL

Sample collection

1. Rinse out the mouth twice with water thoroughly.
2. Collect the tissue by rolling the swab firmly on the inside of the cheek.

Buccal swab Lysate Preparation

1. To a 2 ml microcentrifuge tube, add **1 ml** of **Saliva Stabilization Buffer**.
2. Cut the sample containing portion of the swab and place it inside the tube.
3. Vortex the tube for 30 seconds and incubate at RT for 10 minutes.
4. Transfer the supernatant to a new 1.5 ml microcentrifuge tube.
5. In order to collect the remaining cells in the swab, add **500µl of Saliva Stabilization Buffer** to the tube and vortex it for 30 seconds.
6. Pool both supernatants in a 1.5 ml tube and centrifuge at 14,000 rpm for 5 minutes at RT.
7. Carefully discard the supernatant and add **250 µl of Saliva Lysis Buffer.**
8. Resuspend the pellet in the buffer (*thoroughly by pipette mixing*).
9. Add **10 µl of Proteinase K** and mix the contents by vortex.
10. Incubate the tube at **56ºC** for 1 hour.
11. Add **10 µl of RNase A** and mix the contents by vortex.
12. Incubate at room temperature for 15 minutes.
13. Centrifuge at 14,000 rpm for 5 minutes at RT.
14. Transfer the supernatant to a fresh microcentrifuge tube.

DNA Binding and Washing

15. To the lysate, add **175 µl** of **Saliva MagNa Mix**. Gently mix the contents by inverting the tube for 10 - 12 times. Incubate at RT for 5 minutes.
16. Place the tube on MagNa Stand for 5 minutes.
17. Carefully discard the supernatant without removing the tube from MagNa Stand (*Make sure that the pellet is not disturbed*).
18. Add **200 µl** of **Saliva Wash Buffer 1**, remove the tube from MagNa Stand and resuspend the pellets by pipette mixing for about 5 - 8 times (*Ensure complete dispersion of the particles*).
19. Place the tube back on MagNa Stand until the solution becomes clear (*30 sec - 1 min*).
20. Carefully discard the supernatant without removing the tube from MagNa Stand (*Make sure that the pellet is not disturbed*).
21. Add **200 µl** of **Saliva Wash Buffer 2** and gently invert mix without removing the tube from MagNa Stand for 5 - 6 times to wash the pellet (*surface wash only*).
22. Discard the supernatant without disturbing the pellet.
23. Repeat steps 21 - 22.
24. Air dry the pellet without removing the tube from MagNa Stand at RT for 10 - 15 minutes.

Note: Do not over dry the pellet.

Elute DNA

25. After drying, remove the tube from MagNa Stand.
26. Add **25 µl** of **Saliva Elution Buffer** and resuspend the pellet by pipette mixing 10 -12 times (*Ensure complete dispersion of particles*).
27. Incubate at **56ºC for 10 minutes** with intermittent tapping.
28. Place the tube on MagNa Stand for 5 minutes or until the solution gets cleared.

29. Carefully transfer the supernatant containing DNA to a sterile microcentrifuge tube, without removing the tube from MagNa Stand. Make sure that the pellet is not disturbed.
30. Discard the MagNa particles.

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

Downstream applications

DNA obtained from this method is ready to use for any downstream application without any further precipitation or purification step.

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.
	Age of sample used	Use fresh saliva sample or sample stored at - 20^{o}C (< 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 MagNa particles.
	Improper elution	Completely resuspend the MagNa particles in elution buffer before incubation at 56^{0}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 MagNa particles 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.