PBMC DNA EXTRACTION

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 - 8ºC.

PROTOCOL

Isolation of PBMCs

Note: PBMCs can be prepared using any standard protocol. The protocol given below use Histopaque - 1077.

1. Take **2 ml of fresh blood** in 15 ml sterile centrifuge tube and dilute equally with 2ml of 1X PBS at room temperature and mix well by pipette.
2. Take **3 ml of Histopaque - 1077** in separate 15 ml sterile centrifuge tube.
3. Gently layer 4 ml of diluted blood on top of Histopaque, by tilting the tube and adding the diluted blood sample to the sides of the tube.
4. Centrifuge the mixture at **400g for 30 mins at 18°C** *without applying brake*.
5. Discard the upper plasma layer.
6. Carefully separate and transfer the PBMC layer from the buffy coat, into a new 15ml centrifuge tube and add 5 ml of 1X PBS.
7. Centrifuge at **400g** for **10 mins at 18°C** *with brake*.
8. Discard the supernatant.
9. Resuspend the pellet by pipette mixing in 5 ml of 1X PBS and repeat steps 6 - 7.
10. Resuspend the pellet in 1 ml of 1X PBS and transfer to a 1.5 ml sterile microcentrifuge tube.

PBMCs DNA extraction

Lysate Preparation

1. Centrifuge PBMCs at 10000 rpm for 5 mins at room temperature and discard the supernatant.

2. Add **250 µl of Blood Lysis Buffer 2** to the PBMC cell pellet and resuspend thoroughly by pipette mixing.

Note: For better dispersion, use 200 µl pipette for re-suspending the pellet.

3. Add **10 µl of Proteinase K** and mix by pipetting.
4. Incubate at **56°C for 10 mins**.
5. Transfer the lysate to a new microcentrifuge tube.

DNA Binding and Washing

Note: Ensure that the MagNa Mix is properly mixed prior to use by vortexing

6. To the lysate, add **175 µl of MagNa Mix** and invert mix for about 10 - 12 times and incubate at room temperature for 5 mins.
7. Place the tube on MagNa Stand for 5 mins and then discard the supernatant without removing the tube from MagNa Stand.
8. Add **250 µl of Blood Wash Buffer 1**.
9. Remove the tube from MagNa Stand, resuspend the pellet by proper pipetting till the pellet is completely dispersed.

Note: For better dispersion, use 200 µl pipette for re-suspending the pellet.

10. Place the tubes again on MagNa Stand until the solution gets cleared and discard the clear supernatant.
11. Add **250 µl of Blood Wash Buffer 2** to the tube without removing them from MagNa Stand.
12. Invert mix the tubes for 5 - 6 times and discard the supernatant without removing the tube from MagNa Stand.
13. Repeat steps 11 - 12.
14. Air dry the pellet at room temperature for 10 - 15 mins.

Note: Do not over dry the pellet.

15. To the dried pellet, add **50 µl of Elution Buffer.**
16. Remove the tube from the MagNa Stand, disperse the pellet thoroughly by pipette mixing. *Ensure complete dispersion of particles.*
17. Incubate at **56°C for 5 mins** with intermittent tapping.

18. Place the tube on MagNa Stand for 5 minutes or until the solution gets cleared.
19. Carefully transfer the extracted DNA to a sterile microcentrifuge tube, without removing the tube from MagNa Stand. Make sure that the pellet is not disturbed.
20. 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 then 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

Observation	Possible causes	Suggested Solution
Low DNA yield or Poor Quality	PBMCs not isolated properly	Carefully layer the diluted DNA over Histopaque. If layering is not proper, buffy coat will not be formed.
		Use fresh heparinized blood for isolation of PBMCs. Do not use old/frozen samples.
		Ensure the centrifuge was done at 18ºC only.
	Incomplete Lysis	Use suggested amount of Proteinase K for the specified time.
		Ensure proper re-suspension of pellet while adding the lysis buffers.
		Make sure that the lysis incubation temperature and time is followed correctly.
	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.
	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 56^0C for elution.
	Ethanol is not added	Add 100% ethanol to wash buffers prior to

	to wash buffers	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.