TISSUE/CELL LINE DNA EXTRACTION KIT

Kit Components

Components	Storage
Tissue/Cell Line Lysis Buffer	RT
Proteinase K	2 - 8°C
Proteinase K Buffer	2 - 8°C
RNase A	2 - 8°C
Tissue/Cell Line MagNa Mix	RT
Tissue/Cell Line Wash Buffer 1	RT
Tissue/Cell Line Wash Buffer 2	RT
Tissue/Cell Line Elution Buffer	2 - 8°C
MagNa Stand (optional)	RT

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 Preparation

- **Recommended amount of tissue** for DNA extraction
  - Muscle/Heart: 50 - 60 mg
  - Lungs/Liver/Spleen/Brain/Kidney/Pancreas: 10 - 15 mg
  - Mouse/Rat Tail: 25 - 30 mg
  - Human tumor tissues: < 10 mg
- **Tissue Homogenization**

  Mince the tissue completely using a sterile surgical blade, transfer into a sterile 1.5 ml micro centrifuge tube and weigh the minced tissue as given in the above recommendation. Proceed to tissue lysate preparation.

- **Pre-treatment of Cell Lines (For extraction from Cell lines only)**

1. Add required volume of cells (cell density of $0.5*10^6$ cells) to a 1.5 ml micro centrifuge tube.
2. Centrifuge the sample at 6000 rpm for 5 minutes at room temperature.
3. Add **200 µl of 1X PBS** and resuspend the pellet, centrifuge at 6000rpm for 5 minutes and discard the supernatant.
4. Repeat step 3.
5. Proceed to tissue lysate preparation.

- **Processing of ethanol fixed tissues**

1. Weigh approximately **50 mg** of ethanol fixed tissue.
2. Mince the tissue using a sterile surgical blade.
3. Transfer the minced tissue to a DNase free 1.5 ml microcentrifuge tube.
4. Add 500µl of Nuclease Free Water or PBS. Tap mix the contents.
5. Centrifuge the tube at 8000 rpm for 3 minutes and discard the supernatant.
6. Repeat steps 4 -5.
7. Add another 500 µl of Nuclease Free Water or PBS and tap mix the contents.
8. Incubate at room temperature for 30 minutes.
9. Centrifuge the tube at 8000rpm for 3 minutes.
10. Discard the supernatant and proceed to the tissue lysate preparation.

Tissue Lysate Preparation

1. Add **750 µl of Tissue/Cell Line Lysis Buffer** to the sample.
2. Add **20 µl of RNase A**, vortex the tube for 30 seconds and incubate at room temperature for 15 minutes.
3. Add **20 µl of Proteinase K** and mix by vortexing the tube for 30 seconds.
4. Incubate the samples at 56ºC until the lysate appears clear (*with 1 - 2 times pipetting in between*).

Note: The lysis time varies with the type, storage and amount of tissue used. For soft tissues, lysis is completed within 1 hour and for hard tissues 2 - 8 hours/until lysate

clears. For ethanol preserved tissues, the lysis step might not be completed within 1 hour. You may need to wait till the tissue is completely lysed.

5. After incubation, mix the lysate completely by pipetting and centrifuge the tube at 14000 rpm for 5 minutes at room temperature.
6. Transfer the supernatant to a fresh 1.5 ml micro centrifuge tube.

DNA Binding & Washing

Note: Vortex the Tissue/Cell Line MagNa Mix to ensure complete dispersion of the particles.

7. Add **450 µl of Tissue/Cell Line MagNa Mix** to the lysate and mix by inverting the tube 6 - 8 times. Incubate at room temperature for 5 minutes.
8. Place the tube on the **MagNa Stand** for 5 minutes.
9. Carefully discard the supernatant without removing the tube from MagNa Stand. (*Make sure that the pellet is not disturbed*).
10. Add **250 µl of Tissue/Cell Line Wash Buffer 1** to the tube and remove it from the MagNa Stand.
11. Resuspend the pellet by pipette mixing for about 8 - 10 times (*ensure complete dispersion of the particles*).

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

12. Place the tube back on MagNa Stand until the solution becomes clear (*30secs – 1min*).
13. Carefully discard the supernatant without removing the tube from MagNa Stand (*Make sure that the pellet is not disturbed*).
14. Add **500 µl of Tissue/Cell Line Wash Buffer 2**, gently invert mix the tube without removing from MagNa Stand for about 5 - 6 times (*surface wash only*).
15. Discard the supernatant without removing from the MagNa Stand.
16. Repeat steps 14 - 15.
17. Air dry the pellet without removing the tube from MagNa Stand at room temperature for 10 minutes.

Note: Do not over dry the pellet.

DNA Elution

18. After drying, remove the tube from MagNa Stand.
19. Add **50 – 100 µl of Tissue/Cell Line Elution Buffer** to the tube and resuspend the pellet thoroughly by pipette mixing 10 - 12 times (*ensure complete dispersion of particles*).
20. Incubate at 56°C for 5 minutes with intermittent tapping.
21. Place the tube on MagNa Stand for 5 minutes or until the solution appears clear.
22. Carefully transfer the supernatant containing the DNA to a sterile 1.5 ml micro centrifuge tube, without removing the tube from MagNa Stand. (*Make sure that the pellet is not disturbed*).
23. 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	Incomplete Lysis	Verify whether the tissue sample has lot of fat.
		Check the age of the tissue and ensure that the tissue taken was stored in proper conditions (-80^{o}C).
		Ensure that the recommended amount of tissue is weighed correctly and minced completely.
		Use suggested amount of Proteinase K for the specified time.
		Pipette mix properly during lysis incubation period for complete lysis.
		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 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.
RNA contamination	RNase A not added	Add RNase A as per the protocol.