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XpressDNA Tissue/Cell line Kit

Protocol for isolation of high quality, intact genomic DNA from fresh, frozen, and ethanol preserved mammalian tissues and cell lines.

Process Flow

Kit Contents

Components	Storage Conditions	Shipping Conditions
Tissue/Cell Line Lysis Buffer	RT	RT
Proteinase K	2 - 8°C	RT
Proteinase K Buffer	2 - 8°C	RT
RNase A	2 - 8°C	RT
Tissue/Cell Line MagNa Mix	RT	RT
Tissue/Cell Line Wash Buffer 1	RT	RT
Tissue/Cell Line Wash Buffer 2	RT	RT
Tissue/Cell Line 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.
4. 1X PBS

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 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

1. Mince the tissue completely using a sterile surgical blade.
2. Transfer it into a sterile 1.5 ml tube and weigh the minced tissue **as per the recommendation above**.
3. 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 tube.
2. Centrifuge the sample at 6000 rpm for 5 minutes at RT.
3. Add 200 µl of 1X PBS and resuspend the pellet, centrifuge at 6000 rpm 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 tube.
4. Add 500 µl 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 RT for 30 minutes.
9. Centrifuge the tube at 8000 rpm for 3 minutes.
10. Discard the supernatant and proceed to the tissue lysate preparation.

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Protocol

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 RT for 15 minutes. 3. Add **20 µl of Proteinase K** and vortex mix the tube for 30 seconds. 4. Incubate the samples at 56°C until the lysate appears clear ***(with intermittent pipetting every 30 minutes)*** ***(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, pipette mix the lysate thoroughly. 6. Centrifuge the tube at 14000 rpm for 5 minutes at RT. 7. Transfer the supernatant to a fresh 1.5 ml tube.

DNA Binding	***(Note: Vortex Magna Mix thoroughly before the next step)*** 8. Add **450 µl of Tissue/Cell Line MagNa Mix** to the lysate and invert mix 6 - 8 times. Incubate at RT for 5 minutes. 9. Place the tube on a MagNa Stand for 5 minutes. 10. Carefully discard the supernatant without removing the tube from the MagNa Stand. Ensure the magnetic nanoparticles are not disturbed.

DNA Washing	11. Add **250 µl of Tissue/Cell Line Wash Buffer 1** to the tube and remove it from the MagNa Stand. 12. Resuspend the magnetic nanoparticles by pipette mixing thoroughly to ensure complete dispersal. ***(Note: For better dispersion, use 200 µl pipette for re-suspending the pellet.)*** 13. Place the tube back on the MagNa Stand for 30 - 60 seconds until the solution becomes clear. 14. Carefully discard the supernatant without removing the tube from the MagNa Stand. Ensure the magnetic nanoparticles are not disturbed. 15. Add **500 µl of Tissue/Cell Line Wash Buffer 2**, gently invert mix the tube 5 - 6 times without removing from the MagNa Stand. ***(Surface wash only).*** 16. Discard the supernatant without removing from the MagNa Stand. 17. Repeat steps **15 - 16.** 18. Air dry the magnetic nanoparticles with the tube on the MagNa Stand at RT for 10 - 15 minutes. Avoid over drying.

DNA Elution	19. After drying, remove the tube from the MagNa Stand. 20. Add 50 - 100 µl of Tissue/Cell Line Elution Buffer to the tube and resuspend the MNPs by pipette mixing thoroughly. 21. Incubate at 56°C for 5 minutes with intermittent tapping. 22. Place the tube back on the MagNa Stand for 5 minutes or until the solution becomes clear. 23. Carefully transfer the supernatant containing the DNA to a sterile 1.5 ml tube, without removing the tube from the MagNa Stand. Ensure the magnetic nanoparticles are not disturbed. 24. Discard the magnetic nanoparticles in the 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

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°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.
	Magnetic nanoparticles 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 MagNa particles 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 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.