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Precellys Tissue DNA Extraction Kit

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Precellys Tissue DNA Extraction Kit

#D05701

For research laboratory use only

User Manual #D11701

Version: 0121

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Introduction and Overview

The Precellys Tissue DNA Extraction Kit provides an easy and rapid method for the isolation of genomic DNA for subsequent DNA analysis such as consistent PCR, real time and end-point, or Southern analysis. Up to 30 mg animal tissue, mouse tail snips, or $5x10^6$ cultured cells can be readily processed. This kit allows for the single or multiple simultaneous processing of samples. There is no need for phenol/chloroform extractions and time-consuming steps are eliminated (e.g. precipitation using isopropanol or ethanol). Purified DNA can be directly used for most sensitive downstream applications including qPCR, Southern blotting, restriction enzyme digestion, DNA-seq, arrays, and methylation analysis…

Description of protocols

Different protocols in this handbook provide detailed instructions to use the Precellys Tissue DNA Extraction Kit for purification of total DNA.

The protocol "**Tissue DNA Protocol**" is for use with the Precellys Tissue DNA Extraction Kit for purification of DNA from animal tissues, rodent tails not included.

The protocol "**Cultured Cells Protocol**" is for use with the Precellys Tissue DNA Extraction Kit for purification of DNA from cultured animal or human cells.

The protocol "**Mouse Tail Snips Protocol**" is for use with the Precellys Tissue DNA Extraction Kit, for purification of

DNA from rodent tail snips.

All protocols can be realized by using the "**Vacuum Protocol**".

Kit Contents

The Precellys Tissue DNA Extraction Kit is available in 2 sizes:

- D05701.5 ea for 5 extraction prep
- D05701.50 ea for 50 extraction prep

Designation	Quantity per kit	
	D05701.5 ea	D05701.50 ea
DNA Mini Columns	5	50
2 mL Collection Tubes	10	100
Binding Buffer	5 mL	20 mL
Tissue Lysis Buffer	5 mL	20 mL
DNA Wash Buffer I	5 mL	25 mL
DNA Wash Buffer II	2.5 mL	25 mL
Elution Buffer	15 mL	30 mL
Proteinase K Solution	150 µL	1.5 mL
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Storage and Stability

All Precellys Tissue DNA Extraction Kit components are guaranteed until the expiry date stated on the package, when stored as follows:

- Proteinase K Solution can be stored at room temperature for up to 6 months upon receipt. For long-term storage (>6 months), store at 2-8°C.
- Store all other components at room temperature (22-25°C).

Check buffers for precipitates before use. Redissolve any precipitates by warming to 37°C.

Precaution for use

Users are recommended to carefully read all instructions for use before starting work.

- For research laboratory use only
- Not for human diagnostic use
- Do not pipet liquids by mouth
- Do not use kit components beyond the expiration date
- Do not eat, drink or smoke in area where kit reagents are handled
- Avoid splashing

Wearing lab gloves, laboratory coat and eye protection glasses is recommended when assaying kit materials and samples.

Materials and equipment required to be supplied by user

In addition to standard laboratory equipment, the following materials are required:

- Precellys tubes adapted to the type of samples targeted (see section «Recommended Precellys protocols»)
- Tabletop microcentrifuge capable of 13,000 x g
- Nuclease-free 1.5 mL microcentrifuge tubes
- Shaking water baths, heat blocks, or incubators capable of 55-70°C
- Vortexer
- 100% ethanol
- 100% isopropanol
- PBS (for cultured cells)
- Vacuum Manifold (for Vacuum protocol only)
- Optional: RNase stock solution (100 mg/mL)

Preparing the reagents

Dilute **DNA Wash Buffer I** with 100% isopropanol as follows and store at room temperature.

Kit	100% isopropanol to be added
D05701.5 ea	2 mL
D05701.50 ea	10 ml

Dilute **DNA Wash Buffer II** with 100% ethanol as follows and store at room temperature.

Kit	100% ethanol to be added
D05701.5 ea	10 mL
D05701.50 ea	100 ml

Check buffers for precipitation before use. Redissolve any precipitates by warming to 37°C.

Recommended Precellys protocols

Species	Sample type	Precellys lysing kit	Precellys Evolution Speed (rpm)	Precellys 24 Speed (rpm)	Recommended program
Mouse	Heart	CK28R 2 mL	8500	6500	3 cycles of 10 s 30 s break between cycles
Mouse	Liver	CK14 2mL	8000	6000	1 cycle of 10 s No break
Mouse	Brain	CK14 2mL	8000	6000	1 cycle of 15 s No break
Mouse	Lung	CK28R 2 mL	8500	6500	3 cycles of 15 s 15 s break between cycles

Mouse	Muscle	CK28R 2 mL	8500	6500	3 to 6 cycles of 20 s 15 s break between cycles
Rabbit	Eye	CK14 2mL	8200	6500	3 cycles of 30 s 30 s break between cycles
Mouse	Cartilage tumor	CK28R 2 mL	8500	6500	3 cycles of 15 s 10 s break between cycles
Mouse	Bone	MK28R 2 mL	8500	6500	6 to 8 cycles of 10 s 15 s break between cycles
Mouse	Tail clip	CKMix	8500	6500	3 cycles of 20 s 15 s break between cycles
-	Cultured cells	CK14	7500	5000	2 cycles of 20 s 15 s break between cycles

Illustrated protocol

Sample homogenization

Lysis

Adjust Binding Conditions

Bind

Wash 3X

Dry

Elute

Before Starting

Before starting any following protocols:

- Set water baths, heat blocks, or incubators to 70°C
- Prepare DNA Wash Buffer I and DNA Wash Buffer II according to the directions in the “Preparing the reagents” section
- Heat Elution Buffer to 70°C
- Chill PBS at 4°C for Cultured Cells Protocol

Tissue DNA Protocol

This method is suitable for the isolation of DNA from up to 30 mg tissue. Yields vary depending on source. The protocol can be scaled up to accommodate larger tissue samples.

1. Mince up to 30 mg tissue and transfer sample to the recommended Precellys lysing kit tube (see section «Recommended Precellys protocols»).

2. Add 200 µl Tissue Lysis Buffer.

3. Add 25 µL Proteinase K Solution. Vortex to mix thoroughly.

4. Use the recommended Precellys protocol to disrupt the tissue based on the nature of the sample.

5. **(Optional step)** Incubate at 55°C in a shaking water bath or heat block.

Note: If a shaking water bath is not available, vortex the sample regularly. Lysis time depends on the amount and type of tissue used. The average time is less than 3 hours.

Optional: certain tissues such as liver tissue have high levels of RNA which will be co-purified with DNA using this kit. While it will not interfere with PCR, the RNA may be removed at this point.

RNAse is not provided in the kit, we recommend following protocol:

a. Add 4 µL RNase A (100 mg/mL) per 30 mg tissue.
b. Let sit at room temperature for 2 minutes.
c. Proceed to Step 6 below

6. Centrifuge at maximum speed (≥10,000 x g) for 5 minutes.

7. Transfer the supernatant to a sterile 1.5 mL microcentrifuge tube. Do not disturb or transfer any of the insoluble pellet or the lysing beads.

8. Add 220 µL Binding Buffer. Vortex to mix thoroughly.

Note: A wispy precipitate may form upon the addition of Binding Buffer. This is does not interfere with DNA recovery.

9. Incubate at 70°C for 10 minutes.

10. Add 220 µL 100% ethanol. Adjust the volume of ethanol required based on the amount of starting material. Vortex to mix thoroughly.

11. Insert a DNA Mini Column into 2 mL Collection Tube.

12. Transfer the entire sample from Step 10 to the DNA Mini Column including any precipitate that may have formed.

13. Centrifuge at maximum speed (≥10,000 x g) for 1 minute.

14. Discard the filtrate and reuse the collection tube.

15. Repeat Steps 12-14 until all of the sample has been transferred to the column.

16. Add 500 µL DNA Wash Buffer I.

Note: DNA Wash Buffer I must be diluted with 100% isopropanol before use. Please refer to "Preparing the reagents" section.

17. Centrifuge at maximum speed for 30 seconds.

18. Discard the filtrate and collection tube.

19. Insert the DNA Mini Column into a new 2 mL Collection Tube.

20. Add 700 µL DNA Wash Buffer II.

Note: DNA Wash Buffer II must be diluted with 100% ethanol before use. Please refer to "Preparing the reagents" section.

21. Centrifuge at maximum speed for 30 seconds.

22. Discard the filtrate and reuse the collection tube.

23. Repeat Steps 20-22 for a second DNA Wash Buffer II wash step.

24. Centrifuge the empty DNA Mini Column at maximum speed for 2 minutes to dry the column.

Note: This step is critical for removal of trace ethanol that may interfere with downstream applications.

25. Transfer the DNA Mini Column into a nuclease-free 1.5 mL microcentrifuge tube.

26. Add 100-200 μL Elution Buffer heated to 70°C.

27. Let sit at room temperature for 2 minutes.

28. Centrifuge at maximum speed for 1 minute.

29. Repeat Steps 26-28 for a second elution step.

Note: Each 200 μL elution will typically yield of 60-70% of the DNA bound to the column. Thus, two elutions will generally yield ~90%. However, increasing the elution volume will reduce the concentration of the final product. To obtain DNA at higher concentrations, elution can be carried out using 50-100 μL Elution Buffer (which slightly reduces overall DNA yield). Volumes lower than 50 μL greatly reduce yields. In some instances, yields may be increased by incubating the column at 70°C (rather than at room temperature) upon the addition of Elution Buffer.

30. Store eluted DNA at -20°C.

Cultured Cells Protocol

This protocol is designed for the rapid isolation of up to 25 µg genomic DNA from up to $5x10^6$ cultured cells.

1. Prepare the cell suspension using one of the following methods:

   a. Frozen cell samples should be thawed before starting this protocol. Pellet the cells by centrifugation. Wash the cells with cold PBS (4°C). Resuspend cells in 200 µL PBS. Proceed to Step 2.

   b. For cells grown in suspension, pellet 5×10^6 by spinning at 1,200 x g in a centrifuge tube. Aspirate and discard the supernatant, and wash the cells once with cold PBS (4°C). Resuspend cells in 200 µL PBS. Proceed to Step 2.

   c. For cells grown in a monolayer, harvest the cell by either using a trypsin treatment or by scraping with a rubber policeman. Wash cells twice with cold PBS (4°C). Resuspend the cells in 200 µL PBS. Proceed to Step 2.

2. Put the resuspend cells in the recommended Precellys lysing kit tube (see section «Recommended Precellys protocols»)

3. Add 25 µL Proteinase K Solution. Vortex to mix thoroughly.

4. Use the recommended Precellys protocol to disrupt the cells based on the nature of the sample.

Note: If a shaking water vortex is not available, vortex the sample

regularly. Lysis time depends on the amount and type of tissue used. The average time is less than 3 hours.

Optional: Cultured cells have high levels of RNA which will be co-purified with DNA using this kit. While it will not interfere with PCR, the RNA may be removed at this point.

RNAse is not provided in the kit, we recommend following protocol:

a. Add 4 µL RNase A (100 mg/mL) per 30 mg tissue.
b. Let sit at room temperature for 2 minutes.
c. Proceed to Step 5 below

5. Centrifuge at maximum speed (≥10,000 x g) for 5 minutes.

6. Transfer the supernatant to a sterile 1.5 mL microcentrifuge tube. Do not disturb or transfer any of the lysing beads.

7. Add 220 µL Binding Buffer. Vortex to mix thoroughly.

Note: A wispy precipitate may form upon the addition of Binding Buffer. This is does not interfere with DNA recovery.

8. Incubate at 70°C for 10 minutes. Briefly vortex the tube once during incubation.

9. Add 220 µL 100% ethanol. Vortex to mix thoroughly.

10. Insert a DNA Mini Column into 2 mL Collection Tube.

11. Transfer the entire sample from Step 9 to the DNA Mini Column including any precipitate that may have formed.

12. Centrifuge at maximum speed (≥10,000 x g) for 1 minute.

13. Discard the filtrate and reuse the collection tube.

14. Add 500 μL DNA Wash Buffer I.

Note: DNA Wash Buffer I must be diluted with 100% isopropanol before use. Please refer to "Preparing the reagents" section.

15. Centrifuge at maximum speed for 30 seconds.

16. Discard the filtrate and collection tube.

17. Insert the DNA Mini Column into a new 2 mL Collection Tube.

18. Add 700 μL DNA Wash Buffer II.

Note: DNA Wash Buffer II must be diluted with 100% ethanol before use. Please refer to "Preparing the reagents" section.

19. Centrifuge at maximum speed for 30 seconds.

20. Discard the filtrate and reuse the collection tube.

21. Repeat Steps 18-20 for a second DNA Wash Buffer II wash step.

22. Centrifuge the empty DNA Mini Column at maximum speed for 2 minutes to dry the column.

Note: This step is critical for removal of trace ethanol that may interfere with downstream applications.

23. Transfer the DNA Mini Column into a nuclease-free 1.5 mL microcentrifuge tube.

24. Add 100-200 µL Elution Buffer heated to 70°C.

25. Let sit at room temperature for 2 minutes.

26. Centrifuge at maximum speed for 1 minute.

27. Repeat Steps 24-26 for a second elution step.

Note: Each 200 µL elution will typically yield of 60-70% of the DNA bound to the column. Thus, two elutions will generally yield ~90%. However, increasing the elution volume will reduce the concentration of the final product. To obtain DNA at higher concentrations, elution can be carried out using 50-100 µL Elution Buffer (which slightly reduces overall DNA yield). Volumes lower than 50 µL greatly reduce yields. In some instances, yields may be increased by incubating the column at 70°C (rather than at room temperature) upon the addition of Elution Buffer.

28. Store eluted DNA at -20°C.

Mouse Tail Snips Protocol

1. Snip two pieces of mouse tail 0.2-0.5 cm in length and place into the recommended Precellys lysing kit tube (see section «Recommended Precellys protocols»).

Note: Follow all regulations regarding the safe and humane treatment of animals. Mice should not be older than 6 weeks as lysis will be more difficult in older animals resulting in suboptimal DNA yields. If possible, obtain tail biopsies at 2-4 weeks and freeze samples at -70°C until DNA is extracted.

2. Add 200 µL Tissue Lysis Buffer.

3. Add 25 µL Proteinase K Solution.

4. Use the recommended Precellys protocol to disrupt the cells based on the nature of the sample.

5. **(Optional step)** Incubate at 55°C in a shaking water bath.

Note: If a shaking water vortex is not available, vortex the sample regularly. Lysis time depends on the amount and type of tissue used. The average time is less than 3 hours.

Optional: Mouse tail snips have low levels of RNA which will be co-purified with DNA using this kit. While it will not interfere with PCR, the RNA may be removed at this point.

RNAse is not provided in the kit, we recommend following protocol:

a. Add 4 µL RNase A (100 mg/mL) per 30 mg tissue.
b. Let sit at room temperature for 2 minutes.
c. Proceed to Step 6 below

6. Centrifuge at maximum speed (≥10,000 x g) for 5 minutes to pellet insoluble tissue debris and hair, and beads.

7. Transfer clear lysate to a sterile 1.5 mL microcentrifuge tube. Do not disturb or transfer any of the insoluble pellet or the lysing beads.

8. Add one volume Binding Buffer and one volume 100% ethanol. Vortex to mix thoroughly.

Example: If you transfer 180 µL cleared lysate, add 180 µL 100% ethanol.

Note: A wispy precipitate may form upon the addition of Binding Buffer. This is does not interfere with DNA recovery.

9. Insert a DNA Mini Column into 2 mL Collection Tube.

10. Transfer the entire sample from Step 8 to the DNA Mini Column including any precipitate that may have formed.

11. Centrifuge at maximum speed (≥10,000 x g) for 1 minute.

12. Discard the filtrate and reuse the collection tube.

13. Add 500 µL DNA Wash Buffer I.

Note: DNA Wash Buffer I must be diluted with 100% isopropanol before use. Please refer to “Preparing the reagents” section.

14. Centrifuge at maximum speed for 30 seconds.

15. Discard the filtrate and collection tube.

16. Insert the DNA Mini Column into a new 2 mL Collection Tube.

17. Add 700 µL DNA Wash Buffer II.

Note: DNA Wash Buffer II must be diluted with 100% ethanol before use. Please refer to “Preparing the reagents” section.

18. Centrifuge at maximum speed for 30 seconds.

19. Discard the filtrate and reuse the collection tube.

20. Repeat Steps 17-19 for a second DNA Wash Buffer II wash step.

21. Centrifuge the empty DNA Mini Column at maximum speed for 2 minutes to dry the column.

Note: This step is critical for removal of trace ethanol that may interfere with downstream applications.

22. Transfer the DNA Mini Column into a nuclease-free 1.5 mL microcentrifuge tube.

23. Add 100-200 µL Elution Buffer heated to 70°C.

24. Let sit at room temperature for 2 minutes.

25. Centrifuge at maximum speed for 1 minute.

26. Repeat Steps 23-25 for a second elution step.

Note: Each 200 µL elution will typically yield of 60-70% of the DNA bound to the column. Thus, two elutions will generally yield ~90%. However, increasing the elution volume will reduce the concentration of the final product. To obtain DNA at higher concentrations, elution can be carried out using 50-100 µL Elution Buffer (which slightly reduces overall DNA yield). Volumes lower than 50 µL greatly reduce yields. In some instances, yields may be increased by incubating the column at 70°C (rather than at room temperature) upon the addition of Elution Buffer.

27. Store eluted DNA at -20°C.

Vacuum Protocol

Recommended Settings

The following is required for use with the Vacuum Protocol:

a) Vacuum Manifold
Compatible Vacuum Manifolds: Qiagen QIAvac24, Sigma AldrichVM20, Promega Vacman®, or manifold with standard Luer connector
b) Vacuum Flask
c) Vacuum Tubing
d) Vacuum Source (review table below for pressure settings)

Conversion from millibars:	Multiply by:
Millimeters of mercury (mm Hg)	0.75
Kilopascals (kPa)	0.1
Inches of mercury (inch Hg)	0.0295
Torrs (Torr)	0.75
Atmospheres (atmos)	0.000987
Pounds per Square Inch (psi)	0.0145

Protocol

Carry out disruption, homogenization, protease digestion, and loading onto the DNA Mini Column as indicated in previous protocols. Instead of continuing with

centrifugation, follow the steps outlined below.

Note: Please read through previous sections of this manual before beginning this protocol paying particular attention to the “Materials and Equipment to be Supplied by User”.

1. Prepare samples by following one of the protocols above:
    a. Tissue protocol: steps 1-10
    b. Cultured cells protocol: steps 1-9
    c. Mouse tail snip protocol: steps 1-8
2. Prepare the vacuum manifold according to manufacturer’s instructions and connect the DNA Mini Column to the manifold.
3. Transfer the entire sample to the DNA Mini Column, including any precipitate that may have formed.
4. Switch on vacuum source to draw the sample through the column.
5. Turn off the vacuum.
6. Add 500 µL DNA Wash Buffer I.

Note: DNA Wash Buffer I must be diluted with 100% isopropanol before use. Please refer to “Preparing the reagents” section.

7. Switch on vacuum source to draw the DNA Wash Buffer I through the column.

8. Turn off the vacuum.

9. Add 700 µL DNA Wash Buffer II.

Note: DNA Wash Buffer II must be diluted with 100% ethanol before use. Please refer to “Preparing the reagents” section.

10. Switch on vacuum source to draw the DNA Wash Buffer II through the column.

11. Turn off the vacuum.

12. Repeat Steps 9-11 for a second DNA Wash Buffer II wash step.

13. Remove the column from the vacuum manifold and transfer to a new 2 mL Collection Tube.

14. Centrifuge the empty DNA Mini Column at maximum speed for 2 minutes to dry the column.

Note: It is important to dry the column membrane before elution. Residual ethanol may interfere with downstream applications.

15. Insert the DNA Mini Column into a nuclease-free 1.5 mL microcentrifuge tube.

16. Add 50-200 µL Elution Buffer heated to 70°C.

Note: Refer to individual protocols for recommended elution volumes

17. Let sit at room temperature for 2 minutes.

18. Centrifuge at maximum speed for 1 minute.

19. Repeat Steps 16-18 for a second elution step.

Note: Each 200 µL elution will typically yield of 60-70% of the DNA bound to the column. Thus, two elutions will generally yield ~90%. However, increasing the elution volume will reduce the concentration of the final product. To obtain DNA at higher concentrations, elution can be carried out using 50-100 µL Elution Buffer (which slightly reduces overall DNA yield). Volumes lower than 50 µL greatly reduce yields. In some instances, yields may be increased by incubating the column at 70°C (rather than at room temperature) upon the addition of Elution Buffer.

20. Store eluted DNA at -20°C.

Yield and Quality of DNA

Determine the absorbance of an appropriate dilution (20- to 50- fold) of the sample at 260 nm and then at 280 nm. The DNA concentration is calculated as follows:

DNA concentration = Absorbance 260 × 50 × (Dilution Factor) µg/mL

A value greater than 1.8 indicates greater than 90% nucleic acid. Alternatively, quantity (as well as quality) can sometimes best be determined by agarose gel/ethidium bromide electrophoresis by comparison to DNA samples of known concentrations.

If necessary the DNA can be concentrated by precipitation. Add sodium chloride to reach a final concentration of 0.1M followed by 2X volumes 100% ethanol. Mix well and incubate at -20°C for 10 minutes. Centrifuge at 10,000 x g

for 15 minutes and aspirate and discard the supernatant. Add 700 μL 70% ethanol and centrifuge at 10,000 x g for 2 minutes. Aspirate and discard the supernatant, air dry the pellet for 2 minutes, and resuspend the DNA in 20 μL sterile deionized water or 10 mM Tris-HCl, pH 8.5.

Source	Sample Amount	Yield (μg)
Whole Blood	200 μL	4-12 μg
Mouse Tail	20 mg	15-25 μg
HeLa Tail	1 x 10^6 cells	5-6 μg
Liver	20 mg	13-22 μg

Troubleshooting

Please use this guide to troubleshoot any problems that may arise.

Problem	Cause	Solution
Clogged column	Incomplete lysis	Modify the Precellys protocol used for tissue homogenization or adjust incubation time
	Sample size is too large	If using more than 30 mg tissue, increase volumes of Proteinase K Solution, Tissue Lysis Buffer, Binding Buffer, and Ethanol
	Sample is viscous	Divide sample into multiple tubes and adjust the volume to 250 µL with Tissue Lysis Buffer.
Low DNA Yield	Incomplete homogenization	Modify the Precellys protocol used for tissue homogenization or adjust incubation time
	Poor elution	Repeat elution with increased elution volume. Incubate columns at 70°C for 5 minutes with Elution Buffer.
	Improper washing	• DNA Wash Buffer I must be diluted with 100% isopropanol before use. • DNA Wash Buffer II must be diluted with 100% ethanol before use.
	Overgrown culture	Overgrown culture contains lysed cells and degraded DNA.
	Sample has low DNA content	Increase starting material and volume of all reagents (Proteinase K Solution, Tissue Lysis Buffer, Binding Buffer, Ethanol) proportionally. Load aliquots of lysate through the column successively.

	Column matrix lost binding capacity during storage	Add 100 µL 3M NaOH to the column prior to loading the sample. Centrifuge at 10,000 x g for 30 seconds. Add 100 µL water to the columns and centrifuge at 10,000 x g for 30 seconds. Discard the filtrate.
Low A_{260}/A_{280} Ratio	Extended centrifugation during elution	Resin from the column may be present in eluate. Avoid centrifugation at speeds higher than specified. The material can be removed from the eluate by centrifugation. It will not interfere with PCR or restriction digests
	Poor cell lysis due to incomplete mixing with Binding Buffer	Repeat the procedure, make sure to vortex the sample thoroughly with Binding Buffer.

Nous contacter

OZYME
Des femmes et des hommes au service de vos recherches

Service client - commande : commande@ozyme.fr
Service technique :
Réactifs : 01 34 60 60 24 - tech@ozyme.fr
Instrumentation : 01 30 85 92 88 - instrum@ozyme.fr