Nuclefy Genomic DNA Purification Kit

Quick guide V1.0

trax LAB SYSTEMS

#TRAXSKU16073

We highly recommend first-time users to get familiar with the complete and detailed extraction process by thoroughly reading the instruction manual. This quick guide covers the most commonly used sample types (tissue, blood, cells) and is designed for experienced users. Protocols for genomic DNA (gDNA) purification from additional sample types (including insects, bacteria, saliva, buccal swabs and yeast) can be found in the instruction manual.

REAGENT STORAGE

Nuclefy Proteinase K and Nuclefy RNase A enzymes must be stored at -15 to -25 °C upon arrival. All other kit components can be stored at room temperature (+15 to +25 °C). Always keep bottles tightly closed and columns as well as collection tubes sealed in the enclosed zip-lock bag. Lysis Buffer and Wash Buffer may form precipitate when stored under cool conditions. Check buffers for precipitate before use and re-dissolve at +37 °C if necessary.

GENERAL NOTES BEFORE GETTING STARTED

- Dilute Nuclefy Wash Buffer concentrate with absolute Ethanol as indicated on bottle label.
- Set a thermal mixer or an alternative heating device to +56 °C
- Pre-heat appropriate volume of Elution Buffer to +60 °C.
- Pre-cool appropriate volume of PBS.

GENOMIC DNA PURIFICATION STEPS

Part 1: Sample Lysis

Part 2: Binding and Elution

Part 1/2: SAMPLE LYSIS

Tissue

Important: Frozen sample material must not thaw. Stabilized and fresh samples should be kept on ice to avoid potential degradation. Work on ice until addition of Tissue Lysis Buffer and Proteinase K.

1. **Weigh in recommended amount of tissue (see table below) and transfer it to a 1.5 ml microcentrifuge tube.**
   Prior tissue homogenization is strongly recommended. Alternatively, tissue must be cut into smallest possible pieces to allow for efficient lysis and to prevent DNA degradation.

Tissue Type	Recommended Input	Maximal Input
Liver	10 mg	10 mg
Kidney	10 mg	10 mg
Spleen	10 mg	10 mg
Heart	10 mg	25 mg
Lung	10 mg	15 mg
Brain	10 mg	12 mg
Muscle	10 mg	25 mg

2. **Add Proteinase K (see table below) and 200 µl Tissue Lysis Buffer. Mix by vigorous vortexing for 10 sec.**
   Avoid time-consuming pipetting steps by preparing a master mix of enzyme and Tissue Lysis Buffer.

Tissue Type	Proteinase K
Brain, Kidney, Skin, Ear Clips	3 µl
All other tissues	10 µl

3. **To reach complete tissue breakup, incubate in a thermal mixer at +56 °C with agitation at full speed (~ 1,400 *rpm*): 5 min if processing homogenized tissue or powder, 5-30 min for tissue pieces.**
   Incubation time can be extended up to 3 h.

4. **Samples > 15 mg: centrifuge at max. speed (> 12,000 x *g*) for 3 min to pellet debris. Transfer supernatant to new 1.5 ml microcentrifuge tube.**

5. **Add 3 µl RNase A and mix by vortexing.**

6. **Incubate in a thermal mixer at +56 °C with agitation at full speed (~ 1,400 *rpm*) for 5 min.**

7. **Continue with Step 1 of Part 2/2: Binding and Elution.**

Whole Blood (non-nucleated)

8. **Transfer 100 µl whole blood to a 1.5 ml microcentrifuge tube.**
   If processing less than 100 µl whole blood, fill the volume up to 100 µl with pre-cooled PBS. Avoid thawing of frozen samples.

9. **Add 10 µl Proteinase K, 3 µl RNase A and 100 µl Blood Lysis Buffer to the sample. Mix immediately by vigorous vortexing for 10 sec.**

10. **Incubate in a thermal mixer at +56 °C with agitation at full speed (~ 1,400 *rpm*) for 5 min.**

11. **Continue with Step 1 of Part 2/2: Binding and Elution.**

Nucleated Red Blood Cells

1. **Transfer 10 µl whole blood to a 1.5 ml microcentrifuge tube.**
   Avoid thawing of frozen samples.

2. **Add 90 µl pre-cooled PBS. Mix by vortexing.**

3. **Add 10 µl Proteinase K and 3 µl RNase A. Mix by vortexing for 5 sec.**

4. **Add 100 µl Blood Lysis Buffer. Mix by vigorous vortexing for 10 sec.**

5. **Incubate in a thermal mixer at +56 °C with agitation at full speed (~ 1,400 *rpm*) for 5 min.**

6. **Continue with Step 1 of Part 2/2: Binding and Elution.**

Cultured cells

1. **Harvest up to 5 x 10^6 cultured cells and centrifuge suspension at > 1,000 x *g* for 1 min. Discard supernatant without touching the pellet.** Leave minimal volume of supernatant in the tube to avoid complete drying of the pellet. When working with frozen cell pellets, thaw by flicking the tube several times before continuing.
2. **Add 100 µl pre-cooled PBS. Resuspend the pellet by carefully pipetting up and down.**
3. **Add 1 µl Proteinase K and 3 µl RNase A. Mix briefly by vortexing.**
4. **Add 100 µl Cell Lysis Buffer. Mix immediately by vigorous vortexing for 10 sec.**
5. **Incubate in a thermal mixer at +56 °C with agitation at full speed (~ 1,400 *rpm*) for 5 min.**
6. **Continue with Step 1 of Part 2/2: Binding and Elution.**

Genomic DNA Cleanup

Important: Depending on purification requirements, two gDNA clean-up protocols are provided.

I. Desalting/Buffer Exchange protocol:

Removal of salts and buffer components (e.g. phenol extraction)

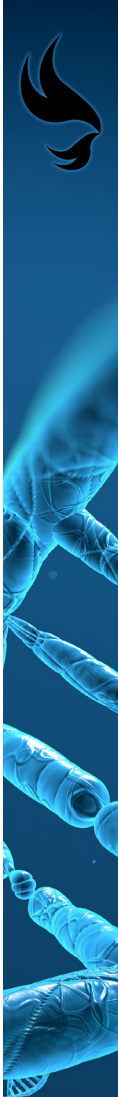
Note: If total DNA input is less than 100 ng, 10 µg/ml of carrier RNA (not supplied) may be added to the Binding Buffer for quantitative DNA retrieval.

1. **Add DNA sample (maximum 200 µl) to a 1.5 ml microcentrifuge tube.**
2. **Mix briefly by vortexing.**
3. **Continue with Step 1 of Part 2/2: Binding and Elution.**

II. Enzymatic protocol:

Removal of proteins and/or RNA

1. **Add DNA sample (maximum 200 µl) to a 1.5 ml microcentrifuge tube.** If processing less than 200 µl of sample, add Tissue Lysis Buffer to bring the total volume to 200 µl.
2. **Mix by vortexing.**
3. **Add 1 µl Proteinase K and 1 µl RNase A. Mix briefly by vortexing.**
4. **Incubate in a thermal mixer at +56 °C with agitation at full speed (~ 1,400 *rpm*) for 5 min.**
5. **Continue with Step 1 of Part 2/2: Binding and Elution.**



Part 2/2: BINDING AND ELUTION

1. **Add 400 µl Binding Buffer to the sample. Mix thoroughly by pulse-vortexing for 5-10 sec.**
   Thorough mixing is essential for optimal results.
2. **Centrifuge briefly to collect any drops from the inside of the lid.**
3. **Insert a Purification Column into a 2 ml Collection Tube.**
4. **Transfer the entire sample (~ 600 µl) to the column.**
   Avoid the transfer of foam and touching the upper column area (risk of salt contamination in eluate).
5. **Centrifuge at 1,000 x *g* for 3 min to bind gDNA. Without emptying collection tube, centrifuge at maximum speed (> 12,000 x *g*) for another 1 min.**
6. **Discard the flow-through and the Collection tube.**
7. **Insert the Purification Column into a new 2 ml Collection Tube.**
8. **Add 500 µl Wash Buffer. Close the cap and invert several times.**
   Inverting the Spin Column prevents salt contamination in eluate.
9. **Centrifuge at maximum speed (> 12,000 x *g*) for 1 min.**
10. **Discard the flow-through.**
    Tip: Tap collection tube on a paper towel to remove any residual buffer.
11. **Re-insert a Purification Column into the Collection Tube.**
12. **Repeat steps 8-11 for a second wash step.**
13. **Centrifuge Purification Column at maximum speed (> 12,000 x *g*) for 2 min.**
    It is important to dry the column before elution. Residual ethanol reduces the elution efficiency and may interfere with downstream applications.
14. **Insert the Purification Column into a nuclease-free 1.5 ml microcentrifuge tube.**
15. **Add 100 µl Elution Buffer (pre-heated to +60 °C) to the matrix and incubate for 1 min.**
    Avoid matrix disruption by any direct contact with tip. Smaller elution volumes can be used (recommended range: 35-100 µl) allowing for more concentrated gDNA, but a reduced yield.
16. **Centrifuge at maximum speed (> 12,000 x *g*) for 1 min.**
17. **Discard the Purification Column. Keep the microcentifuge tube containing eluted gDNA.**
18. **Process eluted gDNA directly or store at +4 °C to +8 °C. For long term storage, store at -80 °C.**

TROUBLESHOOTING

Check the Troubleshooting Guide section in the detailed instruction manual to troubleshoot possible problems that may arise. For further assistance, please contact the technical support staff at support@procomcure.com

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