BROAD INSTITUTE | GENETIC PERTURBATION PLATFORM

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Protocol: Isolation of genomic DNA with NucleoSpin Blood – Mini Kit

Purpose: This protocol is used to extract gDNA from a frozen cell pellet of less than 5e6 cells in 200 uL PBS using the NucleoSpin Blood Mini Kit.

Note prior to starting: *If possible, pellet your cells initially in 200 uL PBS to simplify the protocol.*

Materials:

- Machery Nagel NucleoSpin Blood (Clontech Cat. # 740951)
- RNase A (Clontech, Cat. # 740505), lyophilized, dissolve to 20 mg/ml in H2O
- Absolute ethanol
- A tabletop microcentrifuge that can reach 11,000 x g is required

Getting started:

- Set a water bath to 70°C.
- Preheat Elution Buffer BE on top of a heat block set to 70°C.
- Equilibrate cell pellets to room temperature (15–25°C).
- Prepare Buffer B5 by adding ethanol and prepare Proteinase K by resuspending in Proteinase Buffer according to the kit insert.
- If working with a cell pellet of greater volume than 200 uL, spin down cells and resuspend in 200 uL PBS.

Step 1. Lyse cell pellet

1. Ensure that the cell pellet is resuspended in 200 uL PBS and transfer to a 1.5 mL microcentrifuge tube.
2. Add 25 uL Proteinase K and vortex.
3. Add 1 uL RNase A (20 mg/mL) and vortex.
    - If the cell pellet is still clumpy, incubate at 56°C for 5-20 min and vortex the tube several times to further break up the cells.
4. Add 200 uL of Buffer B3 and vortex the mixture vigorously for 10s.
    - Vigorous mixing is important for DNA yield and purity.
5. Incubate samples at 70°C in the water bath for 10-30 minutes vortexing every 10 minutes until solution is clear.

6. Let the lysate cool down to room temperature (about 5 min) before proceeding with ethanol precipitation.
    - Loading hot lysate onto the columns may result in reduced yields due to evaporation of the ethanol.

Step 2. Precipitate DNA

7. Add 210 uL absolute ethanol to each sample and immediately mix by vortex.
    - High local ethanol concentration must be avoided by immediate mixing.

Step 3. Bind DNA

8. Load all of the lysate onto the provided NucleoSpin Blood Mini Columns.
    - Avoid drops on the rim of the column.
    - The lysate might start to flow through the column even before centrifugation. This will not adversely affect DNA yield or purity.
    - Keep columns in an upright position as liquid may pass through the ventilation slots on the rim of the column even if the caps are closed.
9. Close the tubes and centrifuge 1 min at 11,000 x g. Discard flow-through.

Step 4. Wash silica membrane

10. 1st wash: add 500 uL of Buffer BW to the NucleoSpin Blood Mini column. Centrifuge 1 min at 11,000 x g. Discard flow through.
11. 2nd wash: add 600 uL of Buffer B5. Centrifuge 1 min at 11,000 x g. Discard flow through.
12. Drying step: Centrifuge 1 min at 11,000 x g. Remove the column carefully from the rotor to avoid having the flow-through contact the column outlet. Discard Collection tube.

Step 5. Elute DNA

See Practical Considerations Appendix for alternative elution conditions that may improve gDNA yields.

13. Insert the column into a new 1.5 mL microcentrifuge tube and apply 100 uL of preheated (70°C) Buffer BE directly to the center of the silica membrane.
14. Incubate at room temperature for 5 min (or overnight at 4C).
15. Centrifuge at 11,000 x g for 1 min to elute.

Step 6. Dilute and store gDNA

16. Measure the concentration of gDNA (for example, using NanoDrop or Qubit). If concentration is higher than 200 ng/uL, dilute gDNA with Buffer EB.
    - Calculate the dilutions based on the actual measured volume of eluted DNA as there is always some loss due to the column.
17. Store at 4°C for short term or -20°C for long term.
18. Next, please see: “Protocol for gDNA sample submission for PCR and sequencing.”

Practical Considerations Appendix

RNase:

The original NucleoSpin Blood Mini protocol does not call for the addition of RNAse. However, the presence of RNA contamination in gDNA samples can skew quantification when using absorbance-based methods for determining sample concentrations. Furthermore, large amounts of RNA contamination can have a negative impact on subsequent PCR steps. For these reasons we highly recommend using RNase when performing gDNA isolations.

Elution Volume:

The Genomic DNA from Blood User Manual lists the following elution procedures for highest yield and/or concentration:

- High yield: Perform two elution steps with the volume indicated in the individual protocol. About 90–100% of bound nucleic acid can be eluted
- High concentration: Perform one elution step with 60% of the volume indicated in the individual protocol. Concentration of DNA will be higher than with standard elution Maximal yield of bound nucleic acid is about 80%.
- High yield and high concentration: Apply half the volume of elution buffer as indicated in the individual protocol, incubate for 3 min and centrifuge. Apply a second aliquot of elution buffer, incubate and centrifuge again. Thus, about 85–100% of bound nucleic acid is eluted in the standard elution volume at a high concentration.

Revisions to protocol from previous version:

- Switched from Qiagen to Machery Nagel NucleoSpin kits
- Included RNase A addition to cell lysate to eliminate potential RNA contamination